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LETTER FROM THE EDITOR

Again, during the past year, the Editor and members of the Editorial Board have called upon many of their colleagues to review manuscripts submitted for publication in the JOURNAL. On behalf of the Board, the Editor gratefully acknowledges the contributions of these individuals whose names are listed below.

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At the twenty-third annual meeting of the Central Society for Clinical Research, the Editorial Board and Council of the Society authorized the Editor to select an Associate Editor to assist him in processing the large number of manuscripts now being submitted to the JOURNAL for possible publication. Dr. Matthew Block, Assistant Professor of Medicine, University of Chicago, has accepted this task for 1951.

The Editor again wishes to call attention to the directions which are listed on the inside of the back cover of each issue for the preparation of manuscripts for this JOURNAL. Much time may be lost in processing a paper for publication if these directions are not followed carefully.

—Clayton G. Loosli, M.D.

RWM

THE INQUIRING PHYSICIAN

WESLEY W. SPINK, M.D.*
MINNEAPOLIS, MINN.

IT IS a distinct honor for me to preside over the twenty-third annual meeting of this Society. The Central Society for Clinical Research met for the first time in Chicago in 1928 with Dr. Frank Billings acting as President. Over the intervening years the memership has adhered to the original ojectives of the Society: to cultivate clinical research and to develop younger men. Although a relatively young organization, some measure of tradition has already enveloped its annual proceedings, and one of the customs is for the President to deliver a message of his own choosing. Though the Central Society is a regional group of clinical investigators, approximately a thousand individuals register for the annual fall meeting. This reflects the enthusiasm that many others besides myself have for this meeting. It is a time when new friendships are made and old ones renewed. Furthermore, the scientific program always strikes at a high level, and considerable intellectual stimulus is derived from the excellency of the work of the younger members. Over the years, I have been particularly impressed by the continued enthusiasm of the older men as they return year after year to find out what is new in clinical research. This perennial display of intellectual curiosity has prompted me to speak briefly to you on the subject of "The Inquiring Physician." Most of the men who return here annually have as a part of their daily responsibility the care of sick people. This, of course, is the very keystone of clinical research. I am of the opinion that it makes no difference whether an individual is engaged in so-called academic medicine on a full-time basis or whether he devotes all of his efforts to the private practice of medicine; if he is an Inquiring Physician he will contribute to the sum total of medical knowledge through clinical research. I am well aware of the fact that many well-trained men in the private practice of medicine make no serious attempt to engage in clinical research, although they frequently express wishful thinking along these lines. Various excuses are offered for their failure to carry through with any investigation. They say that they haven't the time, that they lack facilities, or that they do not have the opportunity for sharing in the intellectual stimulus of associates engaged in a similar activity. These are weak explanations. The truth of the matter is that the majority of these men would rather bend their efforts toward making people well and happy. Such men just do not have the time or desire to engage in research. And what more noble purpose can direct human endeavor than the alleviation of pain and the eradication of suffering? I must admit that with some men less ennobling motives spur them on, including that of economic gain. This is not a degrading objective in view of the general pattern of other

Presidential address, Twenty-third Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., Nov. 3 and 4, 1950.

*Department of Medicine, University of Minnesota Medical School.

gainfully employed individuals in society today. There does exist a more fundamental reason why some of the younger men in practice with excellent scientific and clinical training are not advancing medical knowledge. Many of these young men are deterred from engaging in clinical research because of a fatalistic philosophy toward originality that is borne in upon them the moment that they enter medical school. Right at the outset of their training they are exposed to the dogmatic discipline of a basic science, like anatomy, and from then on medical school is a mad scramble to accumulate "facts." As they progress from one area to another, this factual knowledge is handed to them by authorities in their respective fields. At the conclusion of their training, armed with the authoritarianism of medical science, they enter confidently upon the practice of medicine and perform a good job in the care of the patient. Occasionally, beyond the shadow of the medical school, some of these individuals possessing the traits of an Inquiring Physician make clinical observations that do not agree with the facts as presented to them by the authorities. They confirm these observations, which is so essential in clinical investigation. The unfortunate thing is that they fail to report their observations or pursue them further because they are consumed by the fatalistic attitude: who am I as a mere practitioner of medicine to challenge the authority of established medical science? But some individuals have broken through from the extreme periphery of country practice to give new light to the science of medicine.

A phase of clinical research that has interested me, and it has been more than a passing interest, has been the study of the natural history of disease. The Inquiring Physician through the critical observation and study of patients may contribute valuable information to our knowledge of medicine. But his must be a prepared mind. The challenge of naturally occurring phenomena to man has been crystallized by Emerson in his essay on "The Natural History of Intellect," in which he states:

> Each man is a new power in Nature. He holds the keys of the world in his hands. No quality in Nature's vast magazines he cannot touch, no truth he cannot see. Silent, passive, even sulkily, Nature offers every morning her wealth to man. She is immensely rich; he is welcome to her entire goods, but she speaks no word, will not so much as beckon or cough; only this, she is careful to leave all her doors ajar—towers, hall, storeroom and cellar. If he takes her hint and uses her goods she speaks no word; if he blunders and starves she says nothing. To the idle blockhead Nature is poor, sterile, inhospitable. To the gardener her loam is all strawberries, pears, pineapples. To the miller her rivers whirl the wheel and weave carpets and broadcloth. To the sculptor her stone is soft; to the painter her plumbago and marl are pencils and chromes. To the poet all sounds and words are melodies and rhythms. In her hundred-gated Thebes every chamber is a new door.

It is this theme that I wish to develop.

A CONCEPT OF CLINICAL RESEARCH

It is essential that the Inquiring Physician should have a basic concept as to what he means by clinical research. The foundation of all clinical research is the patient. He is Nature's enigma constantly challenging the mind and skill of the physician. The initial stage of clinical research is the observation

of patients, repeated observations, and the documentation of those observations. The observations of others or the analysis of clinical charts cannot act as substitutes. But to stop with observations alone will lead only to sterility. The clinical notes of Hippocrates still retain their authenticity, but even Hippocrates with his astute observations never attempted to unravel the mystery of disease beyond the establishment of shallow theories. The repeated and confirmatory observations of the natural history of a disease should lead the physician to inquire into the fundamental mechanisms by which the disease expresses itself. As a result of reflective thought a multitude of questions will be posed, and out of this will rise a theory, which is so indispensable in the evolution of clinical research. But observation and the development of a hypothesis of disease mechanisms may terminate in erroneous and dangerous conclusions. A theory must be proved or disproved by the experimental method. And by the experimental method it is not necessarily meant that the physician should scurry off to the laboratory and begin the manipulation of chemicals in test tubes or attempt the duplication of the disease process in a lower animal. The experimental method is invoked the moment that the physician attempts to alter the natural history of disease. This occurs when but a single tablet of aspirin is administered at the bedside of a febrile patient. It is reckless to attempt an explanation for the mechanism of disease on the basis of theory alone, particularly when that theory is not based upon long and continued clinical observation. But the greatest error of all in clinical research is to hang on to a theory when experiment will not support it. It was Thomas Huxley who said: "The tragedy of science is the shattering bereavement of seeing a beautiful hypothesis slain by an ugly fact."

The greatest single step in the progress of medical science was made when clinical observation was reinforced by the experimental method. This is a basic concept that is taken for granted today but its evolution occurred only after centuries of mental apathy. Hippocrates was followed by Galen, who at the dawn of the Christian era attempted to introduce the experimental method by means of anatomic dissection. But the mind was ill prepared for this step, and our civilization rested on the dogmatism and vanity of the Galenic tradition for almost fourteen hundred years. Even as late as the seventeenth century the Regius Professor of Medicine at Oxford had the duty of reading twice a week from the works of Hippocrates and Galen. But there were rumblings here and there which were to lead eventually to the cracking of the Galenic discipline. It is well to recall briefly some of the giants responsible for the change in medical thought. In the sixteenth century, Vesalius was among the first to rout the dogma of Galen with his *De Humani Corporis Fabrica.* In the following century, William Harvey clearly established the experimental method with his work on *Motion of the Heart and Blood.* I should like to call attention to the fact that in this volume there are many illustrative cases of circulatory disease which were encountered by Harvey in the beds of St. Bartholomew's Hospital in London. But Hippocrates still had his followers at the time of Harvey, and none was more brilliant than Thomas Syndenham. The Inquiring

Physician should be particularly interested in him because Sydenham believed that every disease had a natural history of its own. The eighteenth century brought among others the great John Hunter, the founder of experimental and surgical pathology. But it was in the nineteenth century that clinical research was to advance by leaps and bounds because clinicians were not content with observation and theory only but pursued the natural history of disease right through to the autopsy table. The French School had its Laënnec, who confirmed his clinical impressions of pulmonary disease by precise anatomic dissection. In England, Richard Bright at Guy's Hospital was delineating disease in a similar manner, along with Thomas Addison. The New Vienna School had the courageous Semmelweis. These men, then, among many others, were clinicians who defined disease more accurately and completely because their shrewd clinical observations were combined with imagination and verified by pathologic anatomy. The nineteenth century was to be closed out by one of the greatest exponents of the experimental method, Claude Bernard, author of the medical classic, *An Introduction to the Study of Experimental Medicine.*

SOME MODERN EXAMPLES OF THE STUDY OF THE NATURAL HISTORY OF DISEASE

The Inquiring Physician of today has a rich heritage in those men who by studying disease at the bedside and then applying the experimental method made significant additions to medical knowledge. But the modern clinician might conclude that Laënnec, Bright, and Addison in their day could study the natural history of disease and thereby advance medicine, but such opportunities are now very limited. He probably is overly impressed by modern medical research with its teams of scientific workers ensconced in lavishly equipped laboratories. Disease processes are extremely complicated, and many basic disciplines must join forces to seek out the hidden mechanism of disease. This does require the presence of highly trained personnel and intricate scientific equipment. This is a part of the evolution of modern scientific inquiry. But there are still opportunities for the clinician to weave together what may first appear to be unrelated clinical phenomena. This synthesis may then provide the spark for an idea so that further observation or experiment will solve some of the mysteries embracing the nature of human disease. It would not be difficult to select many recent examples of this type of clinical research. Only the lack of time prevents an elaboration of this aspect of the subject.

In the north of England, in Yorkshire, a small cluster of villages is nestled in a little valley called Wensleydale. Dr. William Norman Pickles chose to practice in this valley. He was an Inquiring Physician and the isolation of his country practice did not restrain his curiosity. He kept daily records of what he saw among his patients, and just before World War II he published a book summarizing his findings, *Epidemiology in Country Practice.* This small volume has already achieved the stature of a medical classic. The introductory sentence reads: "A gypsy woman driving a caravan into a village in the summer twilight, a sick husband in the caravan, a faulty pump at which she proceeded to wash her dirty linen, and my first and only serious epidemic

of typhoid, left me with a lasting impression of the unique opportunities of the country doctor for the investigation of infectious disease.'' Because Dr. Pickles knew his people and their individual backgrounds, and because they comprised a stable and isolated population, he was able to make a group of basic epidemiologic studies on epidemic catarrhal jaundice, bacillary dysentery, chicken pox and shingles, and other infectious diseases. By 1930, he had established that the incubation period of epidemic jaundice was about one month. It is fascinating to read how he joined together the epidemiologic data on jaundice. His observations have attracted world-wide attention and he was honored by Harvard University in 1948 when he was asked to deliver the Cutter Lecture on Preventive Medicine. ''Let me recommend as a hobby,'' writes Dr. Pickles, ''particularly to those young men entering country practice, this observation of the natural history of epidemic disease.''

One could readily review the achievements of many members of this Society, which have resulted from the study of the natural history of disease. Since time does not permit such a dissertation, I would like to draw your attention momentarily to one of the founders of the Central Society for Clinical Research. His life and his achievements embody many of the traits and accomplishments of the Inquiring Physician. The delightful and stimulating autobiography, *Memoirs of Eighty Years,* by Dr. James B. Herrick of Chicago, describes a medical life that can be divided into three phases: general practice, internal medicine, and cardiology. His most fundamental contribution to clinical research can almost be summarized in two papers presented at meetings of the Association of American Physicians. His first paper was given in 1912 on ''Certain Clinical Features of Sudden Obstruction of the Coronary Arteries,'' and the second presentation in 1918 was on ''Concerning Thrombosis of the Coronary Arteries.'' This unusual man was asked to give another paper before the same august body of physicians at the annual dinner in 1931, and he responded with ''Why I Read Chaucer at Seventy.'' Though he was engaged in the private practice of medicine all of his life, Dr. Herrick found time for teaching and for clinical research. This Society has other illustrative examples of the Inquiring Physician among its founders, and among its younger members.

THE INQUIRING PHYSICIAN HIMSELF

Finally, I would like to devote a moment or two to the underlying philosophy motivating the everyday life of the Inquiring Physician. William Osler in one of the most inspiring of all medical essays, ''The Master-Word in Medicine,'' singled out *WORK* as the key word. A willingness to perform work is a noble and essential virtue for any physician. But, too often, the doctor threshes about with ceaseless physical activity in seeing one patient after another so that he becomes utterly fatigued, and, in addition, he has no time remaining for reflective thinking and restful meditation. Though Charles Lamb remained as only a clerk for thirty-three years with the East India Company, his name is perpetuated as one of the great literary geniuses. Each year he had but a single week for a vacation, and he spent it either with his friend

Coleridge or at an English University. In the evening after the day's work was done he was able to pen the famous *Essays of Elia.* In his essay, "The Superannuated Man," he concluded: "A man can never have too much time to himself. Nor too little to do—I am altogether for the life contemplative."

A requisite virtue for the Inquiring Physician as he pursues his clinical research is that of sustained enthusiasm. Too much of the world's work today is being done by grumbling artisans who rarely, if ever, have experienced the emotional glow that goes with a job well done. One of the most devastating sentences in the English language is to be found in Thoreau's *Walden,* "The mass of men lead lives of quiet desperation." But the Inquiring Physician with a deep and abiding passion for his work will find that his enthusiasm will sustain him through failure as well as success. His efforts will not be motivated by academic honors or prizes, nor by public acclaim. These are but by-products of a deeper yearning for the inner peace that comes to a man who has had work to do and who knows himself when it is accomplished that he has done it well. And when he reaches the end of the road may he have the equanimity of soul that buoyed up the great Laënnec as he lay dying of tuberculosis at the age of 45. He had published his monumental treatise on auscultation, which was met with ridicule and contempt on the part of his contemporaries. It is stated that he received only two letters of praise from the medical world for his effort. But he was still able to say, "I shall consider ample, yea more than sufficient reward for my labor, if it should prove the means by which a single human being is snatched from untimely death."

STUDIES OF COPROPORPHYRIN

V. The Isomer Distribution and Per Diem Excretion of the Urinary Coproporphyrin in Cases of Cirrhosis of the Liver

Cecil James Watson, M.D., Ph.D., Donald Sutherland, M.D., and Violet Hawkinson, B.S.
Minneapolis, Minn.

THE occurrence of increased amounts of porphyrin in the urine in cases of hepatic cirrhosis has been recognized for many years. The pertinent literature has been considered adequately in recent reviews.[1, 2] The preponderant coproporphyrin isomer type in the urine of cirrhotic patients has been determined in a few investigations embracing a total of sixteen cases, the crystalline porphyrin ester being isolated and identified by means of melting point determination. The first of these cases to be studied was an advanced cirrhosis believed to be a residue of a previous cinchophen toxicity.[3] The type I isomer was identified. Coproporphyrin methyl esters were isolated from nine cases of cirrhosis by Dobriner[4] and from two by Vigliani and Libowitzky[5]; Nesbitt[6] obtained them in three cases. In each of these three studies the results were distinctly heterogeneous. Thus Dobriner found the type III isomer in one case labelled as "atrophic" cirrhosis, and in one of "pigment" cirrhosis while two other examples of the latter type, and six other cases of "atrophic" cirrhosis excreted the type I isomer. Nesbitt identified coproporphyrins I and III in two of three cases of "alcoholic" cirrhosis, type III alone in one case, and type I in a case of idiopathic "atrophic" cirrhosis. Vigliani and Libowitzky found the type III isomer in one case, and the type I in another. Further details were not given except that the former of these two also had syphilis and had recently had mercurial therapy.

This fragmentary and conflicting information clearly indicated the need of a more systematic study of coproporphyrin excretion in cases of cirrhosis, so that it could be determined whether the excretion of type I or type III was correlated with etiologic factors, historical features, or other clinical or laboratory data. Such a study first became feasible when a method of isomer analysis applicable to small volumes of urine became available. This method, together with results obtained in normal individuals and in patients with infectious hepatitis, infectious mononucleosis, and obstructive jaundice due to calculus or cancer, was described in previous communications.[7-12] The purpose of the present study was to determine the total urinary coproporphyrin and the isomer distribution in a sufficient number of cases of cirrhosis to permit a comparison of the porphyrin findings with other features.

From the Department of Medicine, University of Minnesota Hospital.

Aided by grants from the John and Mary R. Markle Foundation, New York City, the Division of Research Grants and Fellowships, United States Public Health Service (RG #345), the Office of the Surgeon General, United States Army, under sponsorship of the Commission on Liver Disease, United States Armed Forces Epidemiological Board, and the Medical Research Fund of the Graduate School, University of Minnesota.

Received for publication, Aug. 4, 1950.

MATERIAL AND METHODS

Urine samples collected over a twenty-four hour period were studied in a total of 88 cases of cirrhosis. The present data derive from 270 determinations of the total coproporphyrin in these samples, and 145 estimations of the isomer I and III ratio. The diagnosis of cirrhosis was substantiated in 64 instances by liver biopsy or necropsy. In the others it was believed to be well established on the basis of history and physical and laboratory findings, especially the composite liver function study.[13] The cases were divided, on the basis of the history, into two groups: (1) those in chronic alcoholic individuals, of which there were 42 (Cases 1 to 42); and (2) those in nonalcoholic individuals of which there were 46 (Cases 43 to 88). This division should not be taken to indicate that the latter group consisted solely of teetotalers, but simply that their intake of alcohol, if any, was believed to be relatively insignificant. The individuals of the former group on the contrary, all gave histories indicative of outspoken chronic alcoholism, in many instances seriously interfering with their activities, and often with their diet. Six cases of the nonalcoholic group (Cases 50 and 80 to 84) were proved instances of hemochromatosis.*

In order to facilitate interpretation of the data which will be considered, special attention was given in studying the case records to the following clinical and pathologic features. (1) The presence and approximate severity, on a 0 to 4 plus basis, of ascites, bleeding esophageal varices, spider angiomata, mental disturbance, and foetor hepaticus; (2) the presence of dietary deficiency; (3) the approximate interval, in the alcoholics, since last drinking; (4) the presence and the approximate size or weight of the liver as determined by palpation, peritonescopy, or weight at autopsy; (5) degree of fatty metamorphosis of the liver as determined in biopsy or necropsy material; (6) the approximate severity of the cirrhosis based on the extent of fibrosis, cellular exudate, distortion of lobular architecture, and regenerative activity. This was also classified on a 1 to 4 plus scale, admittedly not more than a rough approximation.

In addition to the total coproporphyrin per twenty-four hours, and the isomer analysis, the following determinations were made in the majority of instances as shown in Table I: hemoglobin in grams per 100 c.c.; reticulocyte percentage; fractional serum bilirubin (1′, or prompt, and total); cephalin-cholesterol flocculation; thymol turbidity; fractional serum proteins; alkaline phosphatase in Bodansky units; total and esterified cholesterol (Sperry-Schönheimer method); urine urobilinogen in milligrams per twenty-four hours, or urine Ehrlich units in two-hour sample, or both; bromsulfalein retention in 45 minutes after 5 mg. per kilogram of body weight, in nonjaundiced subjects only; feces urobilinogen in milligrams per day, or feces Ehrlich units per 100 grams of random sample. These methods have been referred to in more detail elsewhere.[13]

The method of determination of the total coproporphyrin and of the percentage of the type I and III isomers was that of Schwartz and co-workers.[8] Recent studies have revealed that a new and much more sensitive method gives considerably higher UCP (total urinary coproporphyrin) values than the method used in the present study. Evidence will be described in a separate communication revealing that a chromogen of coproporphyrin, hitherto neglected, is responsible in part for the higher values with the new procedure. Thus it is clear that the method upon which the present report is based relates in the main to preformed coproporphyrin; nevertheless the results are entirely valid when compared with the normal data obtained in the same way,[9] nor does this new information in any way affect the validity of the isomer data. Of the two alternative isomer methods described in the paper of Schwartz and associates,[8] procedure A was used in the earlier cases, comprising about one-third of the series, procedure B in those studied more recently. As previously shown, the results obtained with these two procedures are entirely comparable.

As a control for the method of isomer analysis a number of isolations were carried out to permit melting point determinations of the crystalline tetramethyl esters. The method

*We are indebted to Dr. Clement Finch and Dr. Stuart Finch, Department of Medicine, University of Washington, Seattle, Wash., and Peter Bent Brigham Hospital, Boston, Mass., respectively, for providing the essential data and twenty-four hour urine samples from Cases 83 and 84. Both were studied on the medical service of the Peter Bent Brigham Hospital.

TABLE I

Case No.	Initials	Sex	Age	Date	Diet	Approximate interval since last drinking	Diagnosis established	Liver Size or Weight	Fatty liver	Degree of cirrhosis	Ascites	Bleeding varices	Spider nevi	Mental disturbance	Foetor hepaticus	UCP	% type III isomer	Hgb. in Gm./100 cc.	Retic. %	SB 1'	SB T	CC	TT	SA	SG	AP	TC	CE %	UU	UE	B %	FU mg. per day	FE units per 100 gm.
1.	G.O.	♂	44	8-18-44	P	0	A	1.95 k.	1+	3+	3+	0	+	+	+	360	12	7.3	8.2	14.7	20.8			2.6	2.5					1.4			7.3
	Long standing chronic alcoholism. No improvement with methionine. Hyperplastic bone marrow, fatty cirrhosis at autopsy.																																
2.	J.D.	♂	63	11-27-45	N	1½ yr.	B	7 cm.	1+	3+	0	0	+	0	0	308	28	9.0	0.5	0.4	1.4	0	4	3.8	3.2				3.1		21	34	
				3-29-46			A	4.35 k.			1+	0				552		7.8		6.4	10.0	0	3			2.7	124	43		6.6			
	Diffuse cirrhosis; large hepatoma invading right pleural cavity, with hemothorax.																																
3.	K.B.	♂	59	7-29-46	P	1½ yr.	B	--	--	3+	3+	--	+	2+	--	211	58	10.2		1.3	3.2	2+	3	3.6	2.8		138		13.7		35		400
				8-13-46												479	69	10.0		0.9	1.9	3+	2			29.0	118	58			14		
4.	C.B.	♂	59	9-22-46	P	0					2+					223	65			0.3	1.0	1+	2	2.6	3.4		186		3.8		42		
				2-5-47			A	1.18 k.	1+	3+	3+	+																					
	Died out of hospital; autopsy: "severe atrophic cirrhosis."																																
5.	G.B.	♂	37	12-10-45	P	0		4 cm.				0	--	0	0	151	58	4.8	9.0	1.6	3.1	2+	6	2.3	3.8	8.0	118	51	20.0				
				12-23-45			B		2+	2+	2+					171	62	8.0	10.4														
	Chronic alcoholic with poor diet for 15 years. Onset of jaundice and ascites in fall of 1945. Hyperplastic-normoblastic bone marrow.																																
6.	H.B.	♂	45	12-2-43	N	0	C	8 cm.	--	--	0	0	1+	0	0			14.3		0.4	2.2	3+		3.5	3.2		199				38		
				3-7-46	N			0								221	72			0.3	1.3	tr.	1							10.0	16		
	Drinking greatly reduced but not stopped between Dec. '43 and March '46. Dietary history excellent.																																
7.	W.T.	♂	49	2-11-46	P	0		2.38 k.	1+	3+	3+	0	0	2+		518	68	12.3		2.0	4.8	3+	3			10.4	170	54		12.0			
				2-16-46			A									84																	
	Fever, hallucinations, ascites, hematemesis; autopsy: "Portal cirrhosis with chronic alcoholic encephalitis."																																
8.	S.B.	♂	65	10-4-45	P	?	A	3.3 k.	1+	2+	3+	0	+	+	--	190		9.7		1.0	2.4	3+	7	3.2	3.7		320	50		3.4			260
	Ascites and edema 2 months; alcoholism and anorexia many years.																																
9.	C.S.	♂	47	5-11-46	P	0	C	7 cm.	--	--	0	+	--	--	--	245	78	9.4	---	0.3	1.3	1+	1	3.7	2.5	3.0	286	70	12.5	4.6	22		

10.	C.M.	♂	43	8-26-45 5-27-47	P N	0 20 mo.	B	11 cm. NP	3+	2+ 0	0 0	+ 0	+ 0	+ 0	+ 0	466 168	60 49	11.5 15.4		5.4 0.2	10.8 0.6	3+ 0	2 1	2.3	2.7	5.2	136	47	5.0	4.8	5		400
11.	H.D.	♂	57	10-18-46 2-4-47	N N	0 6 mo.	B A	6 cm. 2.35 k.	0 0	2+ 4+	3+ 2+	+ +	0 0	0 3+	0 0	197 185	8 12	13.4 12.4	-- --	0.5 1.0	1.6 3.1	1+ 2+	1 4	3.2 ---	3.6 ---	--- ---	--- ---	-- --	--- 40.5	2.5 18.5	39 42				
	Previous Brucellosis; Br. agglutinins 1:640, + skin test. Died in hepatic coma.																																		
12.	A.M.	♂	54	10-22-46 10-31-46 4-23-47 4-27-47	? F 	3 mo. 21 mo. 	C A	8 cm. 10 cm. 1.24 k.	-- -- 0	-- -- 3+	3+ ? 	0 	3+ 3+ 	0 2+ 	0 + 	385 248 875 375	86 85 25 32	12.0 7.7 4.4	0.5 15.5 25.0	2.8 4.6 7.0	6.5 20.5 20.0	 3+ 3+	2 15 9	 2.4 	 4.8 	 17.0 5.0	 218 118	 27 36	 35.0 10.0	6.0		953	
	Marked hematinemia, macrocytic anemia, hyperplastic-macronormoblastic bone marrow, on last admission. Progressive oliguria, uremia, coma.																																		
13.	J.A.	♀	44	4-25-47 7-16-47	? 	0 0	B A	13 cm. 2.7 k.	3+ 3+	3+ 3+	0 0	0 0	3+ 3+	0 4+	0 0	308	73	12.4		0.9	3.6	3+	4	4.2	3.3	3.8	280	62	20.2						
	Hallucinations; death following a convulsion. 4+ Wassermann.																																		
14.	O.S.	♀	42	1-13-48 2-7-48 2-27-48 10-27-48	P Has F N	0 received 6 wks. 8 mo.	B 860 Has C	10 cm. gm. salt-poor human received large 1 cm.	2+ --	3+ --	+ albumin. amounts IV 0	0 glucose. 0	2+ +	+ 0	0 0	315 414 420 96	72 66 	11.3 8.5 	 7.4 	13.5 16.9 6.6 0.2	29.0 32.7 11.0 1.1	3+ 3+ 3+ 0	7 4.4 7.4 7.0	3.0 5.0 	3.6 2.9 	12.0 3.5 5.4 4.2	206 96 148 	4 18 34 	2.0 0.8 15.5 	 19	 	40 319 	
	Probable Brucellosis in addition to chronic alcoholism and poor diet; agglutinins 1:160--1:640. No apparent improvement following albumin therapy; rapid improvement after intensive intravenous glucose therapy (300 gms. daily with 75 units of insulin, for 23 days. This case has been reported in detail elsewhere.																																		
15.	R.R.	♂	41	1-7-48 1-8-48 1-27-48	F 	2 mo. 	C A	4 cm. 3.0 k.	-- 2+	-- 3+	+ 2+	0 0	2+ 2+	1 3+	+ 2+	294 322	72 80	11.5	2.7	13.9	26.5	4+	5	2.6	5.6	13.4	126	25	4.0						
	Patient a diabetic; nothing suggesting hemochromatosis of homologous serum jaundice. Died in another hospital. (We are indebted to Dr. George V. LeRoy for necropsy report.)																																		
16.	O.D.	♂	55	9-13-45 	F 	12 mo. 	P B	NP 	0 	2+ 	3+ 	0 	0 	0 	0 	41	--	14.3	--	0.2	0.9	1+	2	2.4	2.5	---	---	--	---	1.6	20	---	320		
	Peritoneoscopy revealed a small diffusely nodular liver.																																		
17.	A.R.	♀	59	4-9-43 	N 	? 	P 	NP 	-- 	2+ 	3+ 	0 	0 	0 	0 	198	10	12.4	--	0.2	1.3	2+ 3+	-	2.8	2.7	---	176	--	6.8	---	5.8	---	---		
	Diarrhea for nine years; cholecystenteric fistula first discovered in 1935. Moderate alcoholism: some wine and whiskey, less in past nine years. Had had 10 children.																																		
18.	H.H.	♂	42	9-23-46 9-25-46 11-10-46	P 	2 mo. Marked alcoholism but no history of dietary deficiency.	P B	7 cm. 	-- 	2+ 	2+ 	0 	1+ 	2+ 	0 	62 128 210	 93 90	12.4 	1.4 	0.8 0.3	2.4 1.4	1+ 3+ 2+	4 	2.2 	4.0 	2.2 	204 	62 	 	4.0 	31 29				
19.	H.A.	♂	53	2-8-48 2-13-48 12-7-49	P Biopsy: Adenomata and portal fibrosis. 	4 mo. 	B A	NP 1.46 k.	0 	2+ 	3+ 	0 	2+ 	0 4+ 	+ 	249 291 280	82 80 	11.1 	 	1.0 	3.5 	3+ 3+	3 	2.8 	3.7 	6.0 	140 127	64 57	8.7 17.0						
	Long-standing alcoholism; poor diet; mental aberration. No improvement with choline therapy. Severe cirrhosis at necropsy.																																		

UCP = Total urinary coproporphyrin in γ/24 hours.
SB 1′ = Prompt direct (1′) reacting serum bilirubin in mg. per 100 c.c.
SBT = Total serum bilirubin in mg. per 100 c.c.
CC = Cephalin-cholesterol flocculation test.
TT = Thymol turbidity test.
SA = Serum albumin in Gm. per 100 c.c.
SG = Serum globulin in Gm. per 100 c.c.
AP = Alkaline phosphatase in Bodansky units.
TC = Total cholesterol in mg. per 100 c.c.
CE% = Per cent of esterified cholesterol.
UU = Urinary urobilinogen in mg./24 hours.
UE = Urine Ehrlich units in 2 - 4 P.M. urine sample.
B% = Bromsulfalein retention 45′ after 5 mg. of dye per kilo. of body wt.
FU = Feces urobilinogen in mg. per day (average over four-day period).
FE = Feces Ehrlich units per 100 Gm. of random sample.

(Continued on following pages.)

TABLE I—CONT'D

Case No.	Initials	Sex	Age	Date	Diet	Approximate interval since last drinking	Diagnosis established by	Liver Size or Weight	Fatty liver	Degree of cirrhosis	Ascites	Bleeding varices	Spider nevi	Mental disturbance	Foetor hepaticus	UCP	% type III isomer	Hgb. in gm./100 cc.	Retic. %	SB 1'	SB T	CC	TT	SA	SG	AP	TC	CE %	UU	UE	B %	FU mg. per day	FE units per 100 gm.
20.	W.H.	♂	68	5-9-49 5-15-49 5-16-49	N	6 mo.	B	NP	2+	3+	0	2+	2+	0	0	185 137 220	80 89 92	11.1		0.5	1.4	tr.	2	3.5	2.9							
	Probably no alcohol for more than 6 months.																															
21.	K.B.	♂	41	3-9-46	P	3 wks.	C	3 cm.	--	--	2+	0	3+	2+	+	600	86	10.2	--	7.0	14.4	2+	6	---	---	6.0	166	40	45.0	--	--		64
	Impending delirium tremens. Previous jaundice at age 8. Recent fever, nausea and vomiting. Improved.																															
22.	I.G.	♀	59	11-8-48	N	0	C	6 cm.	--	--	0	0	0	0	0	168	48	12.9	--	0.5	2.5	3+	2	---	---	---	---	--	7.5	--	32	---	
	Steady drinker, relatively mild or minimal evidence of cirrhosis.																															
23.	A.L.	♂	72	10-13-48	P	"17 yrs."	B	NP	0	3+	3+	0	2+	3+	3+	111	43	12.0	--			1+	5	3.6	3.3		202		2.6	--	16	---	
	Long alcoholic history, although patient denied drinking for past 17 years. Died in coma; no autopsy.																				Icterus index = 4.											
24.	H.B.	♂	45	9-28-48	P	2 mo.	L & B	6 cm.	0	2+	tr.	?	3+	2+	0	238	70	13.0	--	0.4	1.2	0	2	4.5	4.2	---	139	--	6.7	--	17		
	Had thrombopenic purpura and splenectomy in 1947, liver biopsy then. Last alcohol about 8-1-48.																															
25.	F.K.	♂	56	11-21-48	P	0	C	6 cm.	--	--	+	0	+	+	0	137	53	12.7	--	0.5	1.0	--	2.5	3.7	3.5	---	161	--	5.5	--	26	---	---
	Has had evidence of multiple vitamin deficiencies; long history of alcoholism.																															
26.	J.K.	♂	54	10-27-48	P	0	L & B	2 cm.	0	3+	+	3+	2+	0	0	153	55	14.4	--	---	---	1+	--	3.8	2.7	---	---	--	4.0	--	--	---	---
	Repeated hematemesis. Thrombopenic purpura in 1947, relieved by splenectomy; liver biopsy then.																															
27.	T.J.	♂	72	11-8-48	N?	1 mo.	A	1.98 k.	0	3+	2+	0	3+	3+	+	183	68	13.8	--	1.6	2.3	4+	4	2.5	2.4	3.1	133	--	---	--	20	---	---
	Long alcoholic history; semi-comatose on admission. History from relatives indicates fairly normal diet.																															
28.	D.R.	♂	57	10-27-48	P	3 mo.	B	6 cm.	3+	2+	+	0	+	2+	0	132	50	17.5	--	---	---	1+	3	3.7	2.8	---	---	--	5.8		26		
	Acute and chronic alcoholism. Biopsy was done 4 months earlier. Has improved on good diet.																															
29.	W.E.	♂	63	9-28-48	P	7 mo.	A	1.56 k.	0	3+	3+	0	3+	4+	0	290	58	11.2	--	---	---	--	3.9	2.5	3.4	14.0	193		3.4		48		
	Spree drinker; no food. Recurrent massive ascites. Severe cirrhosis with splenomegaly.																															
30.	J.V.	♂	61	9-28-48	P	2 mo.	L & B	5 cm.	0	3+	3+	+	0	0	0	166	50	8.2	--	0.2	0.6	0	1.8	---	---	---	166		4.2	---	13		
	Severe spree drinker; also has lues, previous anti-luetic Bi and As. Ascites for past 3 months.																															
31.	E.D.	♂	59	9-28-48	P	3 wks.	C	NP	--	--	3+	0	2+	+	0	1102	80	10.8	--	0.2	1.0	1+	1.6	5.3	3.1	---	---	--	11.8	---	26		
	Mild delirium at time of UCP determination. Ascites for 3 weeks. Severe spree drinker; anorexia, nausea and vomiting.																															
32.	H.L	♂	56	8-25-47	P	5 mo.	A	1.65 k.	2+	2+	2+	2+	4+	3+	2+	--	174	28	11.3	--	4.5	11.1	3+	6	2.5	5.0	9.6	96	31	---	0.8	--	---	---
	Moderately severe alcoholic 15 years. Poor diet several months before onset of jaundice. Fatal hematemesis. Only moderate cirrhosis at autopsy.																															
33.	H.T.	♂	48	1-26-49 3-2-49 3-4-49	P	2 wks.	C	4 cm.	--	--	+	--	0	3+	0	285 74 114	92 96	---	--	1.0	1.8	-- 2+	- 8	--- 2.8	--- 4.8	---	94	47	17.7 4.2				
	Improved with hospitalization and diet. Severe alcoholic 15 years. Hallucinations, chills and trembling. Some abdominal pain.																															

34.	G.F.	♂	46	5-10-49 5-14-49 7-12-49	P	1 wk.	B	NP	2+	3+	4+	2+	3+	0	0	330 530 320	78 92 88	12.6	---	0.4	1.3	1+	7	3.0	3.1	---	230	70	16.0	---	--	---	---
					Severe alcoholism many years. Loss 10 years ago, adequately (?) treated with Bi and As. Wassermann 4+. Marked ascites, no jaundice. Ascites did not recur on 1.0 gm. NaCl or less per day. No improvement with "liver" diet + choline.																												
35.	O.J.	♂	46	6-8-49 6-30-49	N	2 mo.	C	12 cm.	--	--	2+	2+	3+	--	+	297 447	80	8.6 10.3	--- ---	3.5 2.9	10.1 13.4	3+	14	2.4 1.7	5.3 5.8	5.2		16.5				
	Jaundice since Feb. 1949, weakness, ascites, and edema since March. Subjective improvement only in hospital.																														
36.	L.L.	♂	49	3-3-47 4-6-47 6-17-47	P	?	L & B	22.0	1+	3+	0	0	1+	1+	--	176 210 126	31	12.3	0.6	0.4 0.4	1.2 1.3	1+ 2+	11 5.7	3.6	4.2		264 182	70 64		2.0	7 13.5		
	Fever and sweats; slaughterhouse worker and raw milk drinker; Brucella agglutinins and skin test positive. Intermittent jaundice. History vague as to diet and last drinking.																														
37.	E.E.	♂	43	10-31-46	N?	0	B	5 cm.	2+	3+	0	0	3+	0	0	133	58	9.4	1.0	0.2	0.8	0	5	3.4	3.2		154	66	3.5		11		
	Severe alcoholism 20 years. No history of dietary deficiency but evidence of Vitamin A deficiency. Chronic peptic ulcer---gastrectomy in 1941.																														
38.	H.B.	♂	33	7-19-48 7-23-48	P	15 d.	C	12 cm.	--	--	2+	0	3+	0	0	566 454	42	10.0		7.9	16.8		5.3	3.2	3.4		194	21	17.3			63	
	Severe chronic alcoholism; recent delirium tremens; jaundice 3 weeks; previous episodes. Left hospital against advice.																														
39.	T.J.	♂	70	7-3-47 7-27-47	?	4 wks.	B	6 cm.	1+	2+	1+	0	0	1+	0	176 88	11	9.5 9.4	0.8	1.4 0.9	3.6 2.8	3+ 2+	12 13	2.2 2.2	4.7 4.4	2.8	140 180	69 67	91.0 22.0	12.0			
	Diabetic; long-standing alcoholism. Jaundice about 2 months. Biopsy: moderate cirrhosis, increased Fe in liver; mildly fatty.																														
40.	P.H.	♀	42	12-5-47 1-9-48 1-15-48 1-16-48 1-28-48	N	0	B	5 cm.	3+	1+	0	0	0	0	0	180 216 77 150 66	83 74 63 71 60	13.7	1.4	0.2	0.7	1+	2.6	3.7	2.9	7.1	146	62		0.7			
	Tavern worker; drinks beer (whiskey ?). Weight loss and nervousness; marked anorexia 1 month, dietary history normal until then. Biopsy: Markedly fatty liver with early cirrhosis. Marked improvement in hospital.																														
41.	C.S.	♂	42	8-3-49 8-4-49 8-9-49 8-10-49 8-16-49	N	2 mo.	B	9 cm.	2+	3+	2+	?	3+	0	--	313 318 127 519 635	91 90 84 82	10.8		6.8 4.2 3.6	13.8 7.8	1+ 1+	5 7	1.6	4.6	5.5 6.8	134 146	29 74	10.6				60
	Long history of marked alcoholism. "Always good diet." Jaundice and ascites 2 months. Marked unexplained fever in hospital. Biopsy: Fatty cirrhosis; scanty exudate.																														
42.	J.K.	♂	60	4-23-48 5-5-48 5-21-48	P	0	B	8 cm.	2+	2+	1+	0	0	2+	0	255 132	90 81	14.9		0.6	1.6	0	1	4.1	2.5		202	52	92.0	13.6	32		
	Severe alcoholism many years; poor diet only 4 months. Impending delirium tremens. Biopsy: Moderate fatty cirrhosis. Improved with hospitalization and diet.																70														

(Continued on following pages.)

TABLE I—CONT'D

Case No.	Initials	Sex	Age	Date	Diet	Approximate interval since last drinking	Diagnosis established	Liver Size or Weight	Fatty liver	Degree of cirrhosis	Ascites	Bleeding varices	Spider nevi	Mental disturbance	Poetor hepaticus	UCP	% type III isomer	Hgb. in gm./100 cc.	Retic. %	SB 1'	SB T	CC	TT	SA	SG	AP	TC	CE %	UU	UE	B %	FU mg. per day	FE units per 100 gm.
43.	C.L.	♀	67	5-22-44	P	NA	B	8 cm.	0	3+	3+	0	0	0	0	240	9	13.3	---	1.2	2.6	3+	--	2.6	2.7	--	--	--	--	2.5	--	--	284
	Chronic Brucellosis. Fever.					Brucella agglutinins 1:160.		Patient died 10-9-44, no autopsy.										Biopsy: Diffuse portal			fibrosis and lymphocytic exudate.					Non-fatty cirrhosis.						
44.	R.E.	♀	38	2-26-46	N	NA	B	11 cm.	0	3+	0	0	1+	0	0	219		13.8		0.1	0.2	2+	10	3.4	3.4		314	68		0.8	7		
	Generalized arthritis August 1945; jaundice with acholic stools September 1945, 2 months' duration--persistent anorexia,																				large, tender liver, palmar erythema.						Biopsy: Diffuse portal cirrhosis, non-fatty.				
45.	G.K.	♀	56	8-14-47	N	NA	B	5 cm.	0	2+	0	0	0	0	0	80		12.5		0.4	0.8	1+	6	4.5	2.6	37.0	298	32		1.5	17		
	Pigmentation of skin (melanosis) with marked pruritus. Diabetes. No excess of Fe in liver or urine. Bile acids in serum normal. Moderate diffuse portal cirrhosis, non-fatty.																																
46.	M.B.	♀	62	8-14-45	N	NA	B	---	2+	2+	4+	0	1+	0	0	67		6.8	1.8	0.3	0.9	4+	16	2.6	4.0	6.0	94	48	---	---	24	48	
				8-26-45												44		5.0															
	Attack of painless jaundice in 1941. Severe anemia. Palmar erythema.																																
47.	O.C.	♀	47	12-18-45	N	NA	B	10 cm.	0	3+	0	3+	0	0	2+	198	18	6.4	1.1	1.5	2.8	1+	4	2.7	2.5	18.0	176	64	10.0	---	--	---	96
	Patient later had an Eck fistula without improvement. Severe non-fatty, portal cirrhosis.																																
48.	D.M.	♂	33	2-11-48	N	NA	B	12 cm.	0	2+	7	0	2+	--	1+	395	--	10.2	---	12.5	23.5	3+	9.2	3.5	5.2	---	106	27	3.5	---	--	43	---
				2-13-48												137																	
				2-14-48												431																	
				4-29-48				6 cm.			0		3+		2+	300				5.4	11.2	3+	6.3			9.3	174	54	107.0				418
				7-28-48												74				0.4	1.0												
				10-28-48				3 cm.			0					109				0.2	1.0	1+	3.0						2.8		18		
49.	E.M.	♀	63	4-1-47	N	NA	B	3 cm.	0	2+	0	3+	0	0	0	125	10	9.0	1.1	0.1	0.4	0	3	3.4	2.8	---	---	--	0.5	---	5	---	---
				4-10-47												173																	
				6-3-47			A	12 k.	0	3+	2+																						
	Idiopathic diffuse portal cirrhosis with death from bleeding varices.																																
50.	C.K.	♂	62	2-17-46	N	NA	B	9 cm.	0	3+	0	0	0	0	0	230	7	10.1	---	0.9	3.1	3+	4	---	---	6.4	168	61	35.0	---	--	---	88
	Hemochromatosis with diabetes. Biopsy: Extensive hemosiderosis and portal fibrosis.																																
51.	E.P.	♀	25	9-22-46	N	NA					2+	3+	3+	--	--	128	10	9.2		1.0	2.3	2+	4	3.5	3.1	4.0	100	63	---	4.1	--	---	---
				9-25-46												192	7																
				10-21-46			A	2.4 k.						4+	4+	417				8.4	16.1		4	3.3	4.0		76	34					
	Idiopathic diffuse portal cirrhosis with death from bleeding varices.																																
52.	C.E.	♀	74	7-27-46	N	--	B	6 cm.	0	1+	0	0	0	0	0	109	9	8.7			2.1	0	2	3.3	3.0	27.0	312	70		1.5	--		142
	Common duct stones. Liver grossly nodular due to pericholangitis, relatively little fibrosis.																																
53.	I.T.	♀	57	8-26-45	P	NA	B	13 cm.	0	3+	0	2+	0	0	0	120	10	12.3	---	1.5	3.5	2+	30	---	---	36.0	382	10	3.0	---	--	---	120
	Idiopathic cirrhosis; marked palmar erythema. Biopsy: Marked portal cirrhosis, relatively little exudate.																																

54.	B LW	♀	19	10-3-46 6-2-48	N N	NA NA	C C	NP NP	-- --	-- --	0 0	0 0	1+ 1+	0 0	1+ 1+	230 274	9 5	11.3	2.4	2.0	4.4	3+	21	3.1	6.9	11.0	146	55	22	--	--	--	80
	Repeated attacks of jaundice; no known contacts with infectious hepatitis, or other etiology.															371																		
55.	E.S.	♀	53	6-8-46 6-13-46	P	NA	A	NP 1.32 k.	0	3+	0	0	1+	4+	2+	445 371	19	9.9	---	16.3	29.5	4+	10	2.2	6.0	3.6	60	23	46			160	
	Epidemic hepatitis in community in previous year. Diffuse hobnail (portal) cirrhosis. Hepatic encephalopathy.																																	
56.	J.B.	♀	62	5-18-48	P	NA	B	4 cm.	0	2+	0	2+	0	0	?	216	12	12.2	---	3.1	5.5	tr.	4.4	4.0	3.4	21.0	254	61		0.9		---	40
	Poor diet for 2 years; "anemic" for many years. Laparotomy and common duct explorations cirrhosis only.																																	
57.	P.L.	♀	47	10-20-48	N	--	P_B^k	5 cm.	0	2+	0	0	0	0	0	158	16	13.2	3.9	8.3	13.6	tr.	11	---	---	42.0	434	31	19.3	---	--	---	30
	Skin almost black (only melanin on biopsy). Extreme pruritus. Bile acids 8.5 mg. %. Cholangiolitic cirrhosis.																																	
58.	F.W.	♂	14	5-17-47	N	--	C	7 cm.	--	--	0	0	2+	0	1+	179	17	12.5	1.2	1.4	3.6	4+	18	2.7	8.1		106	40		6.1			21
	No definite etiologic factors. Spleen enlarged.																																	
59.	A.C.B.	♀	66	11-8-48	N	--	B	6 cm.	0	2+	0	0	0	0	0	382	67	10.9	---	---	---	4+	7.5	3.5	2.9		350		11.9		24		
	Arthralgia, osteoporosis, palmar erythema, hepatomegaly, moderately advanced portal cirrhosis.																																	
60.	M.B.	♀	23	3-10-46	N	--	A	2.85 k.	0	3+	2+	?	0	1+	0	358	11	3.2	2.0	4.0	8.0	2+	3.0	---	---	18.0	196	57	3.3	---	--	---	30
	Previous cholecystectomy for stones; common duct normal at autopsy; diffuse portal cirrhosis.																																	
61.	M.P.	♀	36	8-7-47 3-10-48	P	--	B	NP	0	3+	0	0	3+	0	0	108 145	12 18	10.9	---	0.2	0.8 1.7	2+ 3+	13 8	2.7 3.0	4.1 4.4		180	70	3.7 8.0		14 9		120
	Probable latent syphilis; 4+ Wassermann.																																	
62.	E.H.	♀	42	2-9-47	N	--	B	NP	0	1+	3+	0	0	0	0	72	20	15.0	1.0	0.3	2.5	0	4	3.9	3.0	4.6	182	73	---	2.0	--	---	---
	Mitral stenosis and insufficiency with long recurring ascites. Mild portal cirrhosis with passive congestion.																																	
63.	S.P.	♀	27	2-25-49 3-9-49	N	--	B	7 cm.	0	2+	0	0	0	0	0	352 245	18 18	9.4	---	15.4	25.0	2+	18	---	---	12.0	155	8.8	5.6				11.2
	(Previous common duct exploration: "Fibrous cord;" cholecystduodenostomy done, functioning on present admission. In November, 1947, TC was 572, AP 29 B.U.																																	
64.	L.S.	♀	26	5-10-44 8-8-44	N	NA	A	1 cm. 1.4 k.	-- 0	-- 3+	-- 1+	-- 0	-- 2+	-- 3+	-- 3+	454 529	8 5	14.6	---	4.3 19.2	7.4 27.3	4+ 4+	--	---	---	---	149 119	---	---	---	--	---	--- 3.0
	In previous years had frequent injections of vaccine for arthritis; gold on one occasion. Died in hepatic coma.																																	
65.	I.V.S.	♀	56	2-2-49 6-20-49	N	NA	B	7 cm.	0	2+	0	0	1+	0	0	155 152	10	--- 11.5	---	5.2 9.0	9.5 15.3	2+ 2+	9 15	2.6	4.2	6.8	208 172	61 53	9.8	16.5			
	Jaundice in 1943 after contact with epidemic jaundice. Cholecystduodenostomy in 1943 for supposed carcinoma of pancreas. Intermittent chills, fever, and jaundice. Died in coma October, 1949; autopsy: Advanced portal cirrhosis; common duct stone.																																	
66.	S.Z.	♂	55	2-9-44	N	--	A	1.5 k.	0	3+	3+	0	0	2+	1+	276	10	13.2	---	0.8	2.2	4+		4.1	1.8		152			10.5			
	Slaughter house worker; Brucella skin test 4+, agglutinins 1:32. Drank 2-3 glasses of beer daily. Appetite and diet always good. Marked portal cellular exudate and fibrosis, without fat.																																	

(Continued on following pages.)

TABLE I—CONT'D

Case No.	Initials	Sex	Age	Date	Diet	Approximate interval since last drinking	Diagnosis established by	Liver Size or Weight	Fatty liver	Degree of cirrhosis	Ascites	Bleeding varices	Spider nevi	Mental disturbance	Foetor hepaticus	UCP	% type III isomer	Hgb. in Gm. / 100 cc.	Retic. %	SB 1'	SB T	CC	TT	SA	SG	AP	TC	CE %	UU	UE	B %	FU mg. per day	PE units per 100 gm.
67.	A.J.	♂	30	6-20-47 6-27-47 7-10-47 7-24-47	N	NA	A	1.78 k.	0	4+	3+	2+	2+	2+	?	419 245 331 568	57 89 96	10.4 11.0	1.0	1.1 1.1	3.1 2.4	3+ 4+	10 8			4.0	176 126	54 45	39.7 80.0 16.0	9.0	31		
	Farmer; raw milk drinker; Brucella agglutinins 1:80, skin test +. Died in hepatic coma preceded by tremors, cog wheel rigidity, ankle clonus, lethargy. Urine: + porphobilinogen, negative uroporphyrin.																																
68.	A.R.	♀	42	7-2-45 1-16-46 10- 7-46 11-2-48	?	NA	B B	4 cm.	0 0	2+ 2+	0	0	0	0	0	89 285 291 174	12 20	9.5	1.0	2.1	13.3	3+	20	2.6	4.4	20.0	250	28	4.8	1.7	16		43
	Recurrent diarrhea and anorexia; pruritus. Common duct normal at operation. Moderate diffuse portal cirrhosis, non-fatty.																																
69.	A.E.	♀	20	5-1-46	N	30 da.	C	NP	--	--	0	0	2+	0	0	159		10.8	---	4.1	7.9	3+	16	2.8	5.9	13.0	178	59	15.0	38.0	--	---	---
	Admitted several drinks about once a month. No definitely etiologic factors. WBC 2000, 65 per cent neutrophiles.																																
70.	B.S.	♀	28	4-18-43	N	NA	C	NP	--	--	2+	0	2+	0	1+	169	6	13.0	2.0	2.7	7.4	3+	--	2.4	2.0	---	---	--	62.0	---	--	306	
	Gradual onset of weakness and pigmentation 3 years earlier. Edema and ascites. Palpable spleen. No etiologic factors.																																
71.	C.O.	♀	46	6-3-43 7-27-45	N	NA	B A	12 cm. 3.0 k.	0 0	2+ 2+	0				1+	220	10	11.5	1.0	1.5	2.9	3+		4.4	3.8		326		52.0				
	since epidemic jaundice at age 13. Chills and fever at times. Death from bleeding esophageal varix. Diffuse, non-fatty portal cirrhosis.										Intermittent jaundice, pruritus, hepato-splenomegaly																						
72.	B.C.	♀	13	8-13-47 9-13-47 6-4-48	N	NA	B	3 cm.	0	3+	0	0	2+	0	0	120 158 144	8	12.0	---	0.8 0.7 3.3	1.5 1.8 6.6	3+ 3+ 3+	28 24 11			18.0 23.0	108 108 96	59 49 30	6.5	3.0			68 208
	Mother and sister had jaundice when patient was 3 1/2. Enlarged liver and spleen, mild jaundice, spider nevi first noted at age 11. Splenectomy in May, 1948; "Banti" spleen, 840 gm. Diffuse portal cirrhosis.																																
73.	S.W.	♀	66	6-8-44 4-2-45	N	NA	B A	10 cm. 1.6 k.	0 0	2+ 3+	0 2+	0 2+	-- --	0 0	-- --	140 147	16	11.0	0.5	8.5	14.0	3+	--	2.8	3.4	34.0	414	31	17.0			44	
	Jaundice and pruritus seven years. Many xanthomata; cholesterol as high as 800 mg. %. Autopsy: Extensive portal cirrhosis; one calcium bilirubinate stone in common duct.																																
74.	T.K.	♀	43	2-23-48	N	NA	B	8 cm.	0	2+	0	0	0	0	0	228	9	12.8	---	2.4	5.1	3+	7	3.6	2.8	26.0	368	45	11.7				180
	Chronic jaundice, pruritus, melanosis. Splenomegaly. Diffuse portal cirrhosis.																																
75.	M.K.	♂	56	5-9-47	N	NA	B	13 cm.	0	2+	0	0	1+	0	0	332	10	12.6	---	0.3	1.2	0	14	2.4	6.1	37.0	104	59	11.0		7		
	Cholecystectomy and common duct drainage 1945 after 6 months of jaundice. Persistent weakness, abdominal pain, enlarged liver. Operation 1947: Common duct stones, grossly cirrhotic liver microscopically pericholangitis and cirrhosis.																																
76.	A.P.	♂	49	10-13-48 1-10-49	N	NA	P & B A	7 cm.	0 0	3+ 4+	1+	0	2+	0	2+	140	12	11.5	---	6.7	13.3	2+	9	2.2	3.9	6.9	184	38	8.4				
	Diabetes, melanosis, hypercholesterolemia (321 mg.% in 1941). Jaundice for many years. No hemosiderosis of skin, urinary sediment, or liver.																																
77.	C.R.	♀	26	4-27-49 5-7-49 6-22-49	N	NA	A	2.5 cm. 1.09 k.			1+	4+	1+			199 302	18	5.4	2.2	2.8 30.0	4.6	3+ 4+	19 14	2.0	4.3	6.4	30	9.4	4.9 11.4				54
	Anemia for 6 years refractory to iron and liver. Slight jaundice for 2 years. Good diet. Fatal hematemesis.																																

78.	T.B.	♀	60	12-6-47 4-2-48	N	NA	B	5 cm.	0	2+	0	0	0	1+	--	266 245	14 12	10.6		11.9	21.9	3+	5.5	2.5	6.0	29.0	162	23	50.2 16.0	28.0			164
	Enlarged liver and spleen. R.U.Q. colic and jaundice for 3 years. Common duct exploration and cholangiogram normal; Biopsy: chronic hepatitis and diffuse portal cirrhosis.																																
79.	E.L.	♀	52	9-18-44	N	NA	B	1 cm.	0	1+	0	0	0	0	1+	130	10	10.5	1.3	2.9	4.3	3+	--	3.6	3.2	22.0	375		12.6				240
	Onset July, 1944, with nausea, vomiting, pruritus; biopsy: cholangiolitic hepatitis and early cirrhosis.																																
80.	L.D.	♂	54	2-27-45 6-13-45	N	NA	B A	9.5 1.9 k.	0 0	3+	0	0	0	0	0	156	13	12.6	0.8	0.4	1.2	2+	--	3.6	2.6		148	68	14.8		10		
	Typical hemochromatosis with diffuse portal cirrhosis, non-fatty.																																
81.	B.U.	♂	43	9-4-47 2-26-49	N	0	B B	11 cm. 9 cm.	0	3+	0	0	3+	0	0	146 244	 16	14.2		0.3	0.6	2+	6.6	3.0	6.0		170	65		0.1	12		
	Moderate alcoholism up to 9-7-47; typical hemochromatosis, with diffuse portal cirrhosis, non-fatty.																																
82.	T.S.	♂	55	4-29-48	P	NA	B	8 cm.	0	2+	0	0	0	0	0	145	10	12.6		0	0.6	tr.	--	3.0	4.6		216	55			7.5		
	Diabetes and generalized pigmentation; typical hemochromatosis with diffuse, non-fatty portal cirrhosis.																																
83.	M.	♂	40	5-49	N	3 yrs.	B	5 cm.	--	--	0	0	1+	0	0	151	11	16.2	2.6		0.6	--	3	4.6	2.3	1.2	150				4	230	
	Hemochromatosis without diabetes. Mild alcoholism until 3 years previously.																																
84.	R	♂	58	6-49	N	0	B	5 cm.	--	--	0	0	2+	0	0	95	10	16.2	2.2		0.9	--	8	4.4	2.4	3.6	157	--	---	---	3.5		
	Hemochromatosis with diabetes. Very mild alcoholism (1-2 glasses of beer per week).																																
85.	E.N.	♀	53	4-2-49	N	NA	L & B	5 cm.	0	2+	0	0	0	0	0	225		12.8		8.0	13.0	1+	11	3.9	4.2	18.0	664	18	5.6				
	Chronic jaundice and pruritus; hyperbilemia. Laparotomy: Cirrhosis, normal cholangiogram. Cholangiolitic type.																																
86.	M.S.	♀	52	11-13-44	N	NA	C	No other lab data on present admission.								99	11																
	Previous history of chronic jaundice, enlarged liver, many injections of vaccine for rheumatoid arthritis; also cinchophen in past.																																
87.	L.I.	♂	56	4-21-48	N	NA	B	4 cm.	0	1+	3+	0	0	0	0	159	10																
	Chronic hepatitis and early diffuse portal cirrhosis.																																
88.	L.D.	♀	8	9-5-45	N	NA	P	2 cm.	--	2+	0	0	0	0	0	240	--	11.5	---	2.0	3.2	4+	16	---	---	6.4	114	70	13.8				79
	Jaundice since age 6. Hepatosplenomegaly. Liver coarsely and diffusely nodular at peritoneoscopy.																																

employed depended upon ether extractions and chromatographic purification, as described elsewhere.[14, 15, 16] The urines from Cases 7, 9, 19, 47, 48, 50, 55, 60, 66, 70, 71, 73, and 74 were used for the isolation of crystalline coproporphyrin methyl esters. Urine from an individual case of infectious hepatitis, not included in the present series, and the pooled concentrates used in quantitative determinations in two series of cases of infectious hepatitis* were subjected to the isolation procedure. These cases were described in paper IV of this series, which did not, however, include the present isolation data. This will be given in the following as a further control on the fluorescence quenching method of isomer analysis.[7, 8] Recrystallizations in all instances were from chloroform-methyl alcohol.

RESULTS

Table I includes the pertinent data for the total coproporphyrin excretion and the isomer distribution, together with the more significant clinical, laboratory, and pathologic features, as already referred to. Much of the clinical and laboratory data accumulated in the study and in particular many of the serial porphyrin determinations have been omitted. The values included are representative and in no instance was there any omission of contradictory or aberrant data. In order to conserve space in Table I the dates are often approximate, but only where this was warranted on the basis of insignificant changes in data.

The total urinary coproporphyrin (UCP) was increased ($>100\gamma$) on the initial determination in 81 of the 88 cases. In two additional cases (Cases 18 and 68, Table I) the initial value was within the normal range but subsequent values were repeatedly increased. Inspection of the data in Table I reveals that there is frequently a rather wide fluctuation in UCP even within short periods of time, the reason for which is not clear. The most remarkable example of this is in Case 7 in which the amount declined from 518 to 84γ in five days. The latter value, however, represented the day before death, and it is quite possible that impaired renal function was responsible for the diminution, although a decreased formation is likewise a possibility. Nesbitt[17] emphasized decrease of UCP in relation to diminishing urinary output in cases of hepatic insufficiency, and renal injury in animals has been shown to cause diminished coproporphyrin excretion.[18] In human uremia the UCP values are very low, commonly less than 10γ per day.† No significant correlation was observed between the extent of increase of the UCP and any of the clinical, pathologic, or laboratory features listed in Table I. In general the more marked increases were encountered in cases with the most outspoken evidence of disease, though a number of exceptions may be noted. While the trend for the UCP values was usually downward in association with clinical improvement, this was not uniform. An example is Case 41 in which definite improvement occurred while the patient was in the hospital, yet the UCP actually increased. It may be of some importance that significant increases of UCP were noted in all instances in which either mental disturbances or foetor hepaticus was recorded. In Case 23, however, the UCP was but 111γ while the foetor hepaticus was recorded as 3+. Spider nevi were encountered in minor degree in one instance (Case 46) without increase of UCP, and in marked degree in another (Case 61) in which

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the increase was relatively slight. Otherwise all cases with spider nevi had significant increases of UCP. Conversely there were many instances of increased UCP values, without nevi. Some degree of increase of the UCP was observed in all instances in which there were bleeding varices, but no correlation with severity was evident. Of more importance from a fundamental standpoint is the fact that the increased UCP in these cases was just as frequently due to the type I as to the type III isomer. This is emphasized because it serves to confirm recent studies[10] which failed to support the older concept that the urinary coproporphyrin may be derived from blood in the gastrointestinal tract.

There was no correlation of the UCP values with the presence or absence of ascites. Marked increases were often noted in cases without ascites; conversely, values in the normal range at least for a time were encountered in five cases exhibiting considerable ascites (Cases 16, 18, 23, 46, and 62). Similarly, no relationship was apparent with liver size or the degree of cirrhosis as estimated histologically. It was somewhat surprising that no correlation was found with the presence or absence of jaundice, or the level of serum bilirubin (Fig. 1). The highest value in the entire series, 1,102γ per twenty-four hours in Case 31, was unassociated with any increase of serum bilirubin. (It is noteworthy that this patient was in a mild delirium tremens at the time of the determination, and that marked elevations have been observed in other cases of alcoholic psychosis.) In Fig. 1 it is seen that there were a number of cases with UCP values between 200 and 350γ per twenty-four hours, with total serum bilirubin less than 1.5 mg. per 100 c.c., and conversely several had markedly elevated serum bilirubin values with UCP between 100 and 175γ per twenty-four hours.

Because of the generally accepted reliability of the bromsulfalein test, it was of particular interest to compare its results with those of the UCP. As mentioned, the bromsulfalein test was limited to nonjaundiced patients, although minor elevations of the serum bilirubin were present in some (Table I). A scattergraph of the bromsulfalein versus the UCP values revealed general agreement as to abnormality, but no correlation otherwise. (Fig. 1.) The two methods were about equally sensitive in the present study, there being four instances in which bromsulfalein retention was 5 per cent or less and in which the UCP was significantly increased, while in three different cases the reverse was true. In one instance both were within the normal range.

Comparison of the UCP data with that for cephalin flocculation and thymol turbidity tests, indicates that the UCP is a somewhat more sensitive indicator of abnormality. Of eighty-seven instances in which the UCP could be compared with either or both of the latter tests, seventy-nine were abnormal ($>100\gamma$ per day). In twenty-six of these the cephalin flocculation test was within normal limits ($<2+$ in twenty-four hours), and in five of the seventy-nine the test was not done. In twenty-five of the seventy-nine the thymol turbidity test was within normal limits (<5 units), and the test was not done in twelve. Conversely, in the eight instances in which the UCP was within normal limits the cephalin flocculation was abnormal in three, normal in four, not done in one; the thymol turbidity was abnormal in four and normal in four.

employed depended upon ether extractions and chromatographic purification, as described elsewhere.[14, 15, 16] The urines from Cases 7, 9, 19, 47, 48, 50, 55, 60, 66, 70, 71, 73, and 74 were used for the isolation of crystalline coproporphyrin methyl esters. Urine from an individual case of infectious hepatitis, not included in the present series, and the pooled concentrates used in quantitative determinations in two series of cases of infectious hepatitis* were subjected to the isolation procedure. These cases were described in paper IV of this series, which did not, however, include the present isolation data. This will be given in the following as a further control on the fluorescence quenching method of isomer analysis.[7, 8] Recrystallizations in all instances were from chloroform-methyl alcohol.

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There was no correlation of the UCP values with the presence or absence of ascites. Marked increases were often noted in cases without ascites; conversely, values in the normal range at least for a time were encountered in five cases exhibiting considerable ascites (Cases 16, 18, 23, 46, and 62). Similarly, no relationship was apparent with liver size or the degree of cirrhosis as estimated histologically. It was somewhat surprising that no correlation was found with the presence or absence of jaundice, or the level of serum bilirubin (Fig. 1). The highest value in the entire series, 1,102γ per twenty-four hours in Case 31, was unassociated with any increase of serum bilirubin. (It is noteworthy that this patient was in a mild delirium tremens at the time of the determination, and that marked elevations have been observed in other cases of alcoholic psychosis.) In Fig. 1 it is seen that there were a number of cases with UCP values between 200 and 350γ per twenty-four hours, with total serum bilirubin less than 1.5 mg. per 100 c.c., and conversely several had markedly elevated serum bilirubin values with UCP between 100 and 175γ per twenty-four hours.

Because of the generally accepted reliability of the bromsulfalein test, it was of particular interest to compare its results with those of the UCP. As mentioned, the bromsulfalein test was limited to nonjaundiced patients, although minor elevations of the serum bilirubin were present in some (Table I). A scattergraph of the bromsulfalein versus the UCP values revealed general agreement as to abnormality, but no correlation otherwise. (Fig. 1.) The two methods were about equally sensitive in the present study, there being four instances in which bromsulfalein retention was 5 per cent or less and in which the UCP was significantly increased, while in three different cases the reverse was true. In one instance both were within the normal range.

Comparison of the UCP data with that for cephalin flocculation and thymol turbidity tests, indicates that the UCP is a somewhat more sensitive indicator of abnormality. Of eighty-seven instances in which the UCP could be compared with either or both of the latter tests, seventy-nine were abnormal ($>100\gamma$ per day). In twenty-six of these the cephalin flocculation test was within normal limits ($<2+$ in twenty-four hours), and in five of the seventy-nine the test was not done. In twenty-five of the seventy-nine the thymol turbidity test was within normal limits (<5 units), and the test was not done in twelve. Conversely, in the eight instances in which the UCP was within normal limits the cephalin flocculation was abnormal in three, normal in four, not done in one; the thymol turbidity was abnormal in four and normal in four.

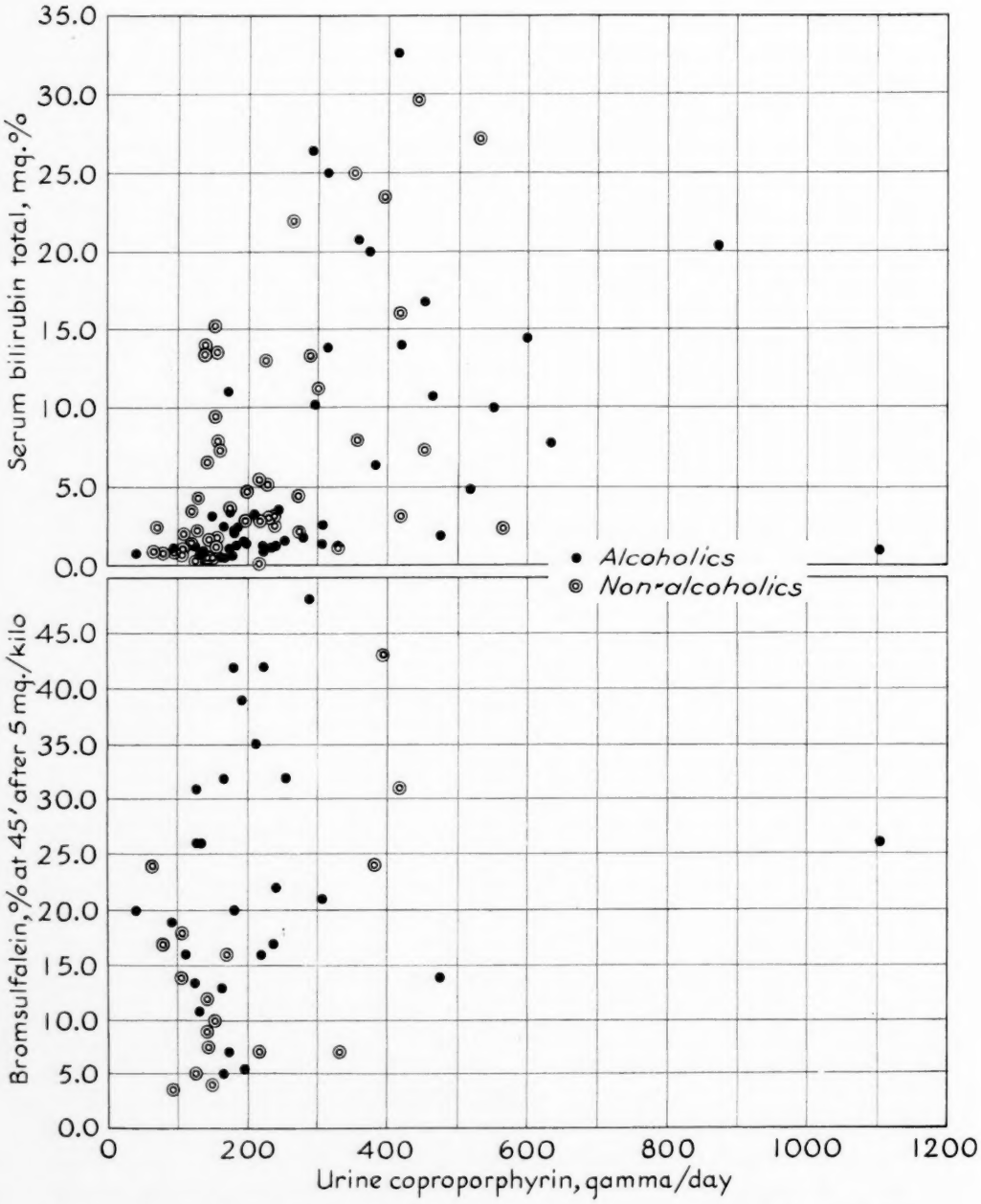

Fig. 1.—Data for total UCP in alcoholic and nonalcoholic cases plotted against total serum bilirubin (above) and bromsulfalein retention (below).

Careful study of the serum albumin and globulin values failed to show any relationship with the UCP. In a number of instances marked increases of the latter were observed when the serum albumin was well within normal limits (Cases 3, 9, 13, 14, 24, 31, and 42), and in some of these the globulin was normal in amount as well. In Case 14 it was of interest that artificial elevation of the

serum albumin to 5.0 Gm. per 100 c.c., by administration of salt-poor human albumin intravenously, was accompanied by actual increases of UCP, although it is doubtful that these were significant at least insofar as any relation to the increasing albumin was concerned.

The data for alkaline phosphatase, as well as total and ester cholesterol, likewise failed to reveal any correlation with the UCP values.

Comparison of the urine urobilinogen per twenty-four hours, or the urine Ehrlich units per two hours, with the UCP reveals fair agreement insofar as abnormality is concerned (Fig. 2) but no quantitative correlation. Values above 400γ of UCP were invariably accompanied by considerable increases of urobilinogen, but in many instances in which the UCP was between 100 and 250γ, even greater increases of urobilinogen were observed. In thirteen cases the value for UU or UE was within the normal range when the UCP was above 100. In but four instances one or the other of these values was significantly increased, with a normal UCP, and in one case both were within the normal range. From this comparison it is evident that while in general there is fair agreement between the two tests, in indicating abnormality, the UCP appears to be somewhat more sensitive.

The data for the isomer distribution in the alcoholic and nonalcoholic individuals are given in Fig. 3. It is readily seen that the alcoholic group as a whole exhibits increases in type III isomer, while the nonalcoholics in the main excrete increased amounts of the type I isomer. The possible reasons for this difference will be discussed below.

The results of the isolations of crystalline esters and melting point determinations are given in Table II. The melting points confirm the isomer data obtained by the fluorescence quenching method.[8]

TABLE II. MELTING POINTS OF CRYSTALLINE COPROPORPHYRIN METHYL ESTERS

CASE NO. (SEE TABLE I)	NUMBER OF RECRYSTALLIZATIONS	MELTING POINT IN °C.
7	2	134
9	2	148 – 150
	3	146 – 48
19	2	136 – 7
47	2	236 – 8
48	1	220 – 2
	2	224 – 6
	3	226 – 30
	4	230 – 4
	5	230 – 4
50	2	230 – 2
55	2	231 – 8
60	2	238 – 9
66	2	240 – 1
70	2	232 – 6
71	2	236 – 7
73	2	238 – 40
74	2	232 – 6
Case G (hepatitis),	2	232 – 4
Pooled Schick* hepatitis cases	2	240 – 2
Pooled DeWitt* hepatitis cases	2	241 – 3
Case 0	2	228 – 30
	3	230 – 2
	4	236 – 7

*Schick and DeWitt U. S. Army General Hospitals, inactivated in 1946.

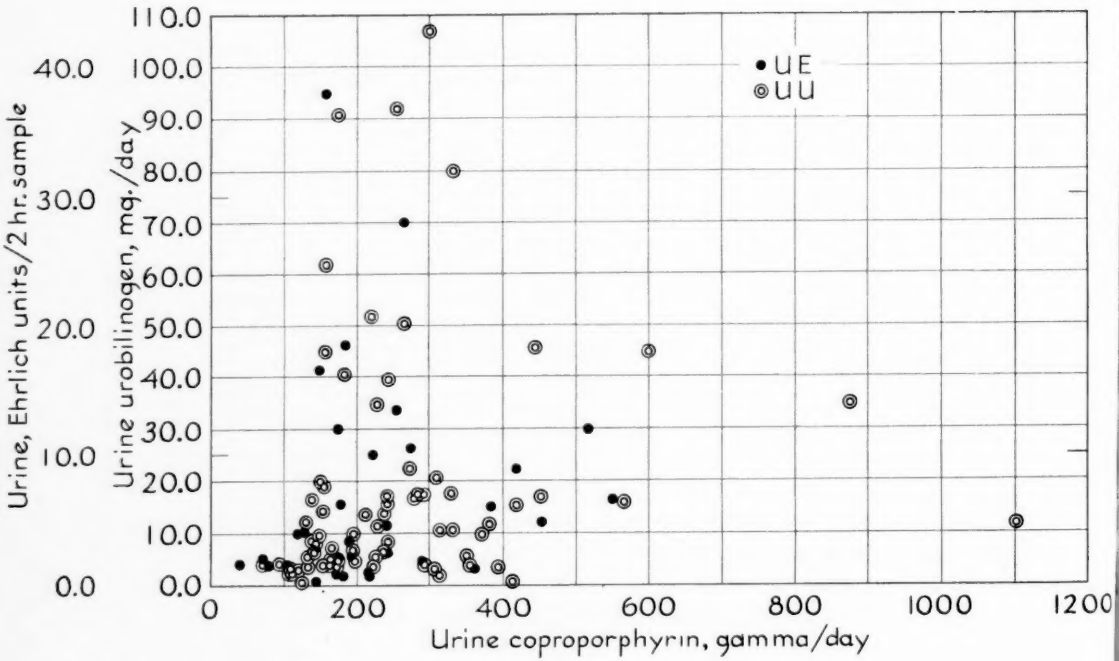

Fig. 2.—Data for total UCP plotted against urine urobilinogen or urine Ehrlich units. The correspondence in level between the two latter values is arbitrary.

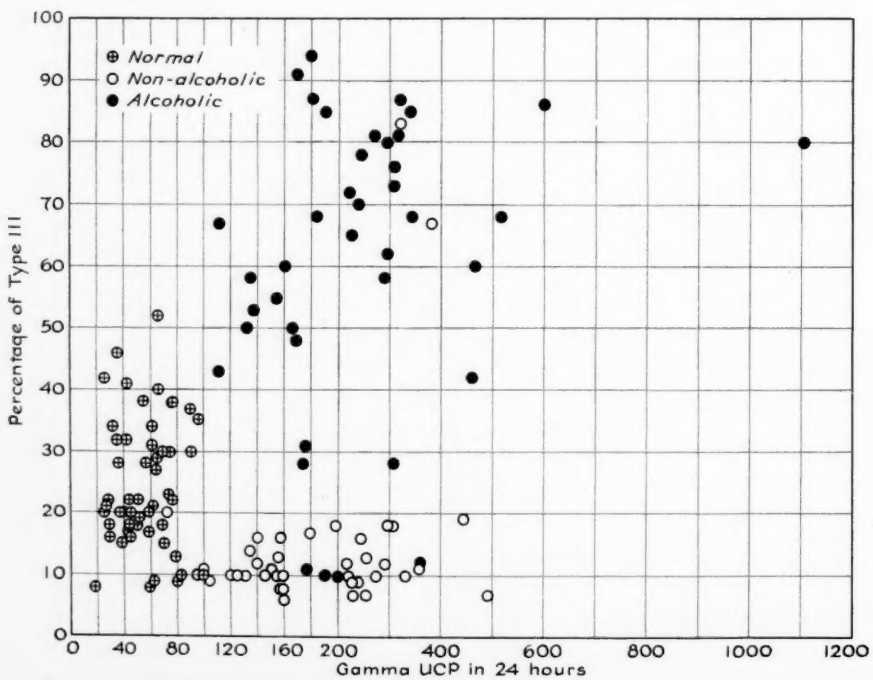

Fig. 3.—Data for total UCP in alcoholic and nonalcoholic cases, plotted against percentage of type III isomer in the same cases. The values plotted are the single values given in Table I or the average of comparable values obtained at the outset in any given instance. Normal data from a previous study[9] employing the same method are included for comparison.

DISCUSSION

Of greater fundamental importance than the increase of the UCP in cases of hepatic cirrhosis is the rather consistent difference in isomer distribution in the "alcoholic" versus the nonalcoholic type, as revealed in the present study. In paper IV of this series[12] it was shown that the increased UCP in obstructive jaundice and infectious hepatitis is the type I isomer, and the relative importance of hepatocellular functional impairment and regurgitation of bile in causing these increases was discussed. With respect to the present results one may ask whether the increase of type III in the alcoholic group, in which there is equally convincing evidence of disturbed liver function, indicates some at least partially reciprocal relationship between the two isomers. It appears, in other words, that formation of the type I isomer in the alcoholic group is often relatively diminished although little can be said as to total amounts until much more data has been obtained for the fecal coproporphyrin in such cases. It is evident, at least, that in the majority of the cases of cirrhosis in alcoholic individuals the type I isomer was either not increased or not as much increased as the outspoken liver functional impairment appeared to warrant. At the same time there were a number of exceptions, Cases 1, 2, 11, 17, 32, 36, and 39, in which, despite a history of alcoholism the type I isomer was preponderant and there are some others in which both isomers are abnormally increased as for example Case 38. The exceptions in the nonalcoholic group, i.e., cases with preponderant type III isomer, were but two, Cases 59 and 67. Careful examination of the various features of these exceptional cases in both groups has failed in general, to explain their deviation. The isomer method is admittedly one that requires considerable skill and care, a research tool rather than a procedure readily adapted to use in a routine biochemical laboratory. The possibility that the exceptions just referred to are due to errors in method cannot be wholly excluded; yet this is believed unlikely in view of the confirmation obtained by isolation and melting point determination of the crystalline esters (Table II). Furthermore there was consistency of the results in the exceptional cases when the determinations were repeated at a subsequent date (Cases 11 and 67). In general, too, it may be noted that the serial isomer analyses have been consistent throughout most of the cases of both groups. The only exception to this, Case 12, is of considerable significance in that during the first period of study the type III isomer was consistently preponderant to a marked degree, while in the second period, six months later, type I was preponderant. This may well be accounted for by the presence of an outspoken hemolytic anemia in the second period. It is seen in Table I that the feces urobilinogen and reticulocytes were markedly increased in association wiith a macrocytic anemia and hematinemia. The increase of the type I isomer under these conditions may probably be ascribed to increased erythropoiesis.[1, 2] It is emphasized that the type III isomer was still considerably increased above the upper normal limit of 34γ per day,[9] being 218 and 120γ per day on the two determinations of this later period. Case 1 in the alcoholic group is another in which increased erythropoiesis may have influenced the isomer ratio; here the reticulocytes were 8.2 per cent but exclusion of bile from

the intestine was almost complete so that no further information could be gained as to a hemolytic factor. It is quite clear, however, that the type III isomer may remain preponderant in spite of an elevated reticulocyte percentage as in Cases 5, 14, and 41, although none of these had such an outspoken hemolytic anemia as Case 2. Case 2, one of the exceptional instances in the alcoholic group, is of special interest because of the long period of probable abstinence and the presence of a large hepatoma with repeated hemothorax as the actual cause of death. Although the reticulocytes were not increased, and the bone marrow was not studied, it seems highly likely that erythropoiesis had been stimulated. Consideration of Case 2 raises two questions: (1) May an isomer ratio which in the earlier stages of an "alcoholic" cirrhosis was preponderantly type III eventually become reversed with prolonged abstinence even though the cirrhosis persists or progresses? (2) Is it not conceivable that some individuals who have been or still are alcoholics may develop a cirrhosis unrelated to alcoholism and dietary deficiency and thus exhibit a preponderance of type I isomer? It is clear that many chronic alcoholics of long standing, even with histories of poor diets, do not develop the slightest evidence of cirrhosis, and one is forced to admit that constitutional or other factors may play an important role in this problem. In any event neither of these questions can be answered at present.

It is noteworthy that all six cases of hemochromatosis (Case 50 and Cases 80 to 84) had preponderance of type I isomer with normal amounts of type III. In Case 81 there had been mild or moderate alcoholism up to within five months of the isomer determination. This is perhaps of some interest with reference to the second of the questions posed in the previous paragraph. The factors responsible for the excessive type III coproporphyrin excretion in cases of "alcoholic" cirrhosis are not yet clear. It seems probable that the alcohol itself is important since, as will be shown in a subsequent paper of this series, acute alcoholism is usually associated with a relatively transitory, often marked increase of the type III isomer in the urine. Chronic alcoholics who stop drinking usually exhibit a normal or not more than slightly increased excretion of coprophyrin III after a variable interval, which rapidly increases again with renewed alcoholism. In Table I it is evident, however, that many of the present patients with cirrhosis had not been drinking at all for relatively long periods, yet exhibited significant or even marked increases of coproporphyrin III in the urine. It would scarcely be acceptable to rely on history in this respect, although in several instances (Cases 3, 12, 15, 18, 19, 20, 24, 29, 35, 41) the dietary history was thought to be fairly reliable. More important, however, are the patients who were observed over a considerable period of time in the hospital, with persistently elevated type III isomer excretion (Cases 14, 18, 33, 34, 40, 42). The cases of "alcoholic" fatty cirrhosis in which marked improvement occurred with abstinence, rest, and a good diet often exhibited a marked diminution of the UCP with persistently elevated percentage of type III isomer (Cases 10, 33, 40). This is particularly interesting from the standpoint of a possible reciprocal relationship such as previously mentioned, when the total UCP drops well into the normal range, the type III, however, remaining elevated to such an extent that type I is necessarily reduced. This is seen in Cases 33 and 40.

In the first of these 96 per cent of 74γ was found to be type III isomer. This might be too high for technical reasons, but it clearly indicates that the amount of type I was greatly diminished. The findings in Case 10 were of much interest because of the long period of abstinence and continuing physical and laboratory improvement. Suffice it to say now that after two years of normal living, the subject's physical and conventional laboratory findings were normal except for slight increase of the twenty-four hour urine urobilinogen. The UCP, however, was still considerably elevated with an abnormally high type III. The biopsy in this case at the outset revealed a rather severe fatty cirrhosis, so it is reasonable to assume that the continued elevation of UCP and type III isomer was related to a latent cirrhosis. Such a sequence of events has now been well established by serial biopsy in other cases.[19, 20]

The possible role of dietary deficiency in producing the increased type III excretion is, of course, an attractive hypothesis, in view of the information which has now accumulated regarding production of fatty cirrhosis in animals fed a (choline) deficient diet. There are several important, but not necessarily conclusive objections: (1) A number of individuals in the alcoholic group (Table I) gave no history whatever of any dietary deficiency (Cases 6, 11, 18, 20, 22, 35, 41), and in some of these the history was well substantiated by family members or friends in close contact with the patient. In spite of this it is recognized that at best too much reliance cannot be placed upon dietary histories. It is recognized too that even with an adequate protein intake, alcoholism may interfere with digestion of protein and absorption of such substances as choline and methionine. (2) Histories of poor diets were evident in a number of cases in the nonalcoholic group, in some for long periods (Cases 43, 53, 55, 56, 61, 82). While it is probable that these were due to anorexia because of the cirrhosis, it is clear at least that none of these had preponderance of type III isomer. (3) The lack of any increase of coproporphyrin III in dogs and rats developing dietary (choline) deficiency fatty cirrhosis.* This objection is questionable, however, in view of the finding* that alcohol alone does not produce type III coproporphyrinuria in these species. This is obviously of considerable importance to the whole question of translating results of experiments on alcohol and cirrhosis in animals to the human disease. In respect to the effect of dietary deficiency alone it would be of much interest to study porphyrin metabolism in the human deficiency states associated with fatty cirrhosis, such as that described by the Gillmans.[21] They noted increased porphyrin in the livers of these individuals, although it was not identified.

Administration of choline and/or methionine has not resulted in any evident change in porphyrin excretion (Cases 1, 4, 14, 15, 34). As will be noted, however, it is conceivable that a considerable period of time is required for adequate improvement in liver cell metabolism, which would make it difficult to determine the effect of dietary substances except over relatively long periods of time. In the present study the administration of choline (for several months in a number of instances) has been without apparent effect on coproporphyrin III excretion.

*Unpublished studies by F. W. Hoffbauer and A. Greenberg.

The possibility has been considered that impurities in alcoholic beverages, such as other organic chemicals or heavy metals, might be responsible for the increased type III isomer excretion, since it is well known that this is regularly induced by chemical or heavy metal toxicity of many types.[1,2] This appears remote, however, in view of the relatively small proportion of chronic alcoholics actually developing cirrhosis, also the relatively transitory character of the increase of coproporphyrin III induced by acute alcoholism, to be discussed in a separate communication.

It should be noted that there are probably multiple sites of formation of the coproporphyrins—the bone marrow, central nervous system, and very likely the liver. In lead poisoning there is much reason to believe that the relatively large amounts of coproporphyrin III excreted in the urine are formed mainly in the bone marrow as an expression of a disturbed hemoglobin synthesis.[22, 23] This appears much less likely for such conditions as poliomyelitis[24] in which the nervous system itself may be the source, or "alcoholic" cirrhosis in which it is quite conceivable that the excessive porphyrin is formed in the damaged liver. These differences require some emphasis as it is clearly unnecessary to seek liver disease as the cause of all excesses of an increased type III isomer excretion.

Considerable evidence has been described suggesting a relationship of the coproporphyrins to cellular metabolism, especially to carbohydrate metabolism in yeast cells or fungi. Fischer and Fink[25] observed a rough correlation in the amount of coproporphyrin synthesized by yeast cells, with their fermentative activity. More recently Kench and Wilkinson[26] have shown that with yeast, alteration of the media is accompanied by a change in proportion of coproporphyrin isomers synthesized; thus in a high glucose medium coproporphyrin I was preponderant, while the type III isomer was larger in amount when the supply of glucose was small. Mayer[27] had observed many years earlier that cyanide inhibits coproporphyrin formation by yeast, indicating its enzymatic character. The possibility exists and deserves exploration that alcohol adversely affects an enzyme system in the liver cells, or perhaps in other cells of the body in such a manner that increased elaboration of coproporphyrin III results. This will be considered more fully in a subsequent paper of this series, but insofar as cirrhosis is concerned it seems wholly feasible that such an injury, often repeated and perhaps abetted by or even requiring a dietary deficiency, might well provide the basis for the type III coproporphyrinuria which so often persists in the cirrhotic individual long after the alcohol has been discontinued. If such an injury could be shown to occur in the liver parenchyma its possible significance for the pathogensis of the cirrhosis would be considerable.

SUMMARY AND CONCLUSIONS

The urinary coproporphyrin (UCP) has been determined in 270 twenty-four hour samples from 88 cases of hepatic cirrhosis, of which 42 were classified as "alcoholic," 46 as nonalcoholic. The isomer ratio (types I and III) was determined 145 times in 79 of the cases. The diagnosis of cirrhosis was confirmed by biopsy or necropsy in 72 cases. The porphyrin data have been studied in relation to the history, physical and pathologic findings, and to the results of a battery of liver function procedures.

Elevated values for the UCP were observed in 83 of the 88 cases. No definite correlation was found between the extent of increase and the clinical features of the disease, although in general, the more severely ill patients excreted large amounts. Increases were observed in all instances in which there were mental disturbances, foetor hepaticus, or bleeding varices.

There was no correlation with the level of total serum bilirubin, nor with that of prompt or indirect reacting fractions. The most marked single increase of the UCP occurred in a patient with a normal serum bilirubin. The UCP was much more sensitive as an index of abnormality. Similarly the UCP was more often abnormal than the twenty-four hour urine urobilinogen, the urine Ehrlich test on two-hour urine samples, or the cephalin flocculation or thymol turbidity tests. Its positivity was approximately equal to that of the bromsulfalein retention test, although there were several instances of disagreement.

The isomer ratio was generally reversed in the alcoholic as compared with the nonalcoholic groups, the type III isomer preponderating in the former and the type I in the latter. Possible explanations are considered both for the excessive type III coproporphyrinuria in the alcoholic cases and for its failure to appear in certain exceptional instances.

REFERENCES

1. Dobriner, K., and Rhoads, C. P.: The Porphyrins in Health and Disease, Physiol. Rev. **20**: 416, 1940.
2. Watson, C. J., and Larson, E. A.: The Urinary Coproporphyrins in Health and Disease, Physiol. Rev. **27**: 478, 1947.
3. Watson, C. J.: Concerning the Naturally Occurring Porphyrins. I. The Isolation of Coproporphyrin I From the Urine in a Case of Cincophen Cirrhosis, J. Clin. Investigation **14**: 106, 1935.
4. Dobriner, K.: Urinary Porphyrins in Disease, J. Biol. Chem. **113**: 1, 1936.
5. Vigliani, E. C., and Libowitzky, H.: Ueber Porphyrine im Harn und im Kot, Klin. Wchnschr. **16**: 1243, 1937.
6. Nesbitt, S.: Excretion of Coproporphyrin in Hepatic Disease. IV. Isolation and Identification of Urinary Coproporphyrin Isomers, Arch. Int. Med. **71**: 483, 1943.
7. Schwartz, S., Hawkinson, V. E., and Watson, C. J.: Quantitative Differentiation of Minute Amounts of the Coproporphyrin Isomers (I and III) Based on Fluorescence Behavior, Science **103**: 338, 1946.
8. Schwartz, S., Hawkinson, V. E., Cohen, S., and Watson, C. J.: A Micromethod for the Quantitative Determination of the Urinary Coproporphyrin Isomers (I and III), J. Biol. Chem. **168**: 133, 1947.
9. Watson, C. J., Hawkinson, V. E., Schwartz, S., and Sutherland, D.: Studies of Coproporphyrin. I. The Per Diem Excretion and Isomer Distribution of Coproporphyrin in Normal Human Urine, J. Clin. Investigation **28**: 447, 1949.
10. Larson, E. A., and Watson, C. J.: Studies of Coproporphyrin. II. An Investigation of the Contribution to the Urinary Coproporphyrin of Hemoglobin and of Bacterial Metabolism in the Intestinal Tract, J. Clin. Investigation **28**: 452, 1949.
11. Watson, C. J., Schwartz, S., Schulze, W., Jacobson, L. O., and Zagaria, R.: Studies of Coproporphyrin. III. Idiopathic Coproporphyrinuria; a Hitherto Unrecognized Form Characterized by Lack of Symptoms in Spite of the Excretion of Large Amounts of Coproporphyrin, J. Clin. Investigation **28**: 465, 1949.
12. Watson, C. J., Hawkinson, V. E., Capps, R. B., and Rappaport, E. M.: Studies of Coproporphyrin. IV. The Per Diem Excretion and Isomer Distribution in the Urine in Infectious Hepatitis, Infectious Mononucleosis, and Mechanical Jaundice, J. Clin. Investigation **28**: 621, 1949.
13. Watson, C. J., and Hoffbauer, F. W.: Liver Function in Hepatitis, Ann. Int. Med. **26**: 813, 1947.
14. Watson, C. J.: Concerning the Naturally Occurring Porphyrins. IV. The Urinary Porphyrin in Lead Poisoning as Contrasted With That Excreted Normally and in Other Diseases, J. Clin. Investigation **15**: 327, 1936.
15. Grinstein, M., Schwartz, S., and Watson, C. J.: Studies of the Uroporphyrins. I. The Purification of Uroporphyrin I and the Nature of Waldenström's Uroporphyrin, as Isolated From Porphyria Material, J. Biol. Chem. **157**: 323, 1945.

16. Watson, C. J., Schwartz, S., and Hawkinson, V. E.: Studies of the Uroporphyrins. II. Further Studies of the Porphyrins of the Urine, Feces, Bile, and Liver in Cases of Porphyria, With Particular Reference to a Waldenström Type Porphyrin Behaving as an Entity on the Tswett Column, J. Biol. Chem. **157**: 345, 1945.
17. Nesbitt, S.: Excretion of Coproporphyrin in Hepatic Disease. III. Urinary Excretion of Coproporphyrin in Hepatic Insufficiency During Episodes Characterized by Neurologic Manifestations, Arch. Int. Med. **71**: 62, 1943.
18. Schwartz, S., and Zagaria, R.: Studies of Porphyrin Metabolism. II. Effects of Metals on Coproporphyrin Excretion. Atomic Energy Commission. MDDC-504.
19. Jones, C. M., and Volwiler, W.: An Evaluation of Various Therapeutic Programs in Patients With Acute Fatty Livers, Tr. A. Am. Physicians **60**: 232, 1947.
20. Buck, R. E.: Observations on Alcoholic Fatty Liver; the Use of Interval Needle Biopsy and Liver Function Tests, J. Lab. & Clin. Med. **33**: 555, 1948.
21. Gillman, J., Gillman, T., and Brenner, S.: Vitamin A and Porphyrin-like Fluorescence in the Livers of Pellagrins, With Special Reference to the Effects of a High Carbohydrate Diet and Methionine, South African J. M. Sc. **10**: 67, 1945.
22. Watson, C. J.: The Erythrocyte Coproporphyrin and its Variation in Respect to Protoporphyrin and Reticulocytes in Certain of the Anemias, Arch. Int. Med. In press.
23. Schmid, R., Schwartz, S., and Watson, C. J.: Porphyrins in the Bone Marrow and Circulating Erythrocytes in Experimental Anemias. To be published.
24. Watson, C. J., Schulze, W. M., Hawkinson, V., and Baker, A. B.: Coproporphyrinuria (Type III) in Acute Poliomyelitis, Proc. Soc. Exper. Biol. & Med. **64**: 73, 1947.
25. Fischer, H., and Fink, H.: Ueber Koproporphyrin-synthese durch Hefe und ihre Beeinflussung. III Mitteil., Koproporphyrinester aus Reinkulturen von Saccharomyces anamensis, Ztschr. f. physiol. Chem. **150**: 243, 1925.
26. Kench, J. E., and Wilkinson, J. F.: Porphyrin Formation by Yeast, Nature **155**: 579, 1945.
27. Mayer, R. M.: Ueber den fermentativen Charakter der Koproporphyrinsynthese in der Hefe. Die zellfreie Koproporphyrinvermehrung, Ztschr. f. physiol. Chem. **179**: 99, 1928.

STUDIES OF COPROPORPHYRIN

VI. The Effect of Alcohol on the Per Diem Excretion and Isomer Distribution of the Urinary Coproporphyrins

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THE preceding communication in this series dealt with the total urinary coproporphyrin (UCP) and the isomer ratio, in cases of hepatic cirrhosis. It was shown that "alcoholic" cirrhosis in general is characterized by an absolute increase in the type III coproporphyrin isomer as contrasted with the increase of type I in the great majority of cases of cirrhosis in nonalcoholic individuals.[1] This finding naturally led to the following question. What is the effect of acute alcoholism on the coproporphyrin excretion in individuals without any evidence of liver disease? The meager literature bearing on this question has been considered in a recent review[2] and and need not be repeated.

MATERIAL AND METHODS

The twenty-four hour urinary coproporphyrin values (UCP) were determined in 108 individuals. Eighty-seven case observations were made within the first ten days following the end of the last alcoholic excess. Most of the subjects in this group were drinking heavily up to twelve to twenty-four hours before the time at which the initial urine collection was started and are representative of acute alcoholism in individuals known to be chronic alcoholics. The average amount of alcohol consumed by this group was at least one pint of whisky per day and the period of drinking usually exceeded two weeks and extended up to nine months.†

Forty-one case observations were made after the first ten "dry" days had elapsed.‡

The effects of alcohol taken orally to the point of drunkenness were observed in a controlled study of five prisoner volunteers,§ all of whom had undoubtedly drunk to excess over long periods of time prior to their incarceration.

The UCP values were determined at least once on all individuals included in the study, and 228 isomer analyses were carried out on samples from 84 individuals.‖ Serial UCP and isomer determinations thus were made in many instances. The method employed was that of Schwartz and associates.[3] Normal ranges of values with this method are as follows:

Total per diem urine coproporphyrin (UCP)	18-99
Per diem coproporphyrin III	3-35
Per diem coproporphyrin I	10-90

From the Department of Medicine, University of Minnesota Hospital.

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‡The material for this part of the study was supplied through the courtesy of the local chapter of Alcoholics Anonymous, with the assistance of Dr. Charles Hewitt.

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16. Watson, C. J., Schwartz, S., and Hawkinson, V. E.: Studies of the Uroporphyrins. II. Further Studies of the Porphyrins of the Urine, Feces, Bile, and Liver in Cases of Porphyria, With Particular Reference to a Waldenström Type Porphyrin Behaving as an Entity on the Tswett Column, J. Biol. Chem. **157:** 345, 1945.
17. Nesbitt, S.: Excretion of Coproporphyrin in Hepatic Disease. III. Urinary Excretion of Coproporphyrin in Hepatic Insufficiency During Episodes Characterized by Neurologic Manifestations, Arch. Int. Med. **71:** 62, 1943.
18. Schwartz, S., and Zagaria, R.: Studies of Porphyrin Metabolism. II. Effects of Metals on Coproporphyrin Excretion. Atomic Energy Commission. MDDC-504.
19. Jones, C. M., and Volwiler, W.: An Evaluation of Various Therapeutic Programs in Patients With Acute Fatty Livers, Tr. A. Am. Physicians **60:** 232, 1947.
20. Buck, R. E.: Observations on Alcoholic Fatty Liver; the Use of Interval Needle Biopsy and Liver Function Tests, J. LAB. & CLIN. MED. **33:** 555, 1948.
21. Gillman, J., Gillman, T., and Brenner, S.: Vitamin A and Porphyrin-like Fluorescence in the Livers of Pellagrins, With Special Reference to the Effects of a High Carbohydrate Diet and Methionine, South African J. M. Sc. **10:** 67, 1945.
22. Watson, C. J.: The Erythrocyte Coproporphyrin and its Variation in Respect to Protoporphyrin and Reticulocytes in Certain of the Anemias, Arch. Int. Med. In press.
23. Schmid, R., Schwartz, S., and Watson, C. J.: Porphyrins in the Bone Marrow and Circulating Erythrocytes in Experimental Anemias. To be published.
24. Watson, C. J., Schulze, W. M., Hawkinson, V., and Baker, A. B.: Coproporphyrinuria (Type III) in Acute Poliomyelitis, Proc. Soc. Exper. Biol. & Med. **64:** 73, 1947.
25. Fischer, H., and Fink, H.: Ueber Koproporphyrin-synthese durch Hefe und ihre Beeinflussung. III Mitteil., Koproporphyrinester aus Reinkulturen von Saccharomyces anamensis, Ztschr. f. physiol. Chem. **150:** 243, 1925.
26. Kench, J. E., and Wilkinson, J. F.: Porphyrin Formation by Yeast, Nature **155:** 579, 1945.
27. Mayer, R. M.: Ueber den fermentativen Charakter der Koproporphyrinsynthese in der Hefe. Die zellfreie Koproporphyrinvermehrung, Ztschr. f. physiol. Chem. **179:** 99, 1928.

STUDIES OF COPROPORPHYRIN

VI. The Effect of Alcohol on the Per Diem Excretion and Isomer Distribution of the Urinary Coproporphyrins

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THE preceding communication in this series dealt with the total urinary coproporphyrin (UCP) and the isomer ratio, in cases of hepatic cirrhosis. It was shown that "alcoholic" cirrhosis in general is characterized by an absolute increase in the type III coproporphyrin isomer as contrasted with the increase of type I in the great majority of cases of cirrhosis in nonalcoholic individuals.[1] This finding naturally led to the following question. What is the effect of acute alcoholism on the coproporphyrin excretion in individuals without any evidence of liver disease? The meager literature bearing on this question has been considered in a recent review[2] and and need not be repeated.

MATERIAL AND METHODS

The twenty-four hour urinary coproporphyrin values (UCP) were determined in 108 individuals. Eighty-seven case observations were made within the first ten days following the end of the last alcoholic excess. Most of the subjects in this group were drinking heavily up to twelve to twenty-four hours before the time at which the initial urine collection was started and are representative of acute alcoholism in individuals known to be chronic alcoholics. The average amount of alcohol consumed by this group was at least one pint of whisky per day and the period of drinking usually exceeded two weeks and extended up to nine months.†

Forty-one case observations were made after the first ten "dry" days had elapsed.‡

The effects of alcohol taken orally to the point of drunkenness were observed in a controlled study of five prisoner volunteers,§ all of whom had undoubtedly drunk to excess over long periods of time prior to their incarceration.

The UCP values were determined at least once on all individuals included in the study, and 228 isomer analyses were carried out on samples from 84 individuals.‖ Serial UCP and isomer determinations thus were made in many instances. The method employed was that of Schwartz and associates.[3] Normal ranges of values with this method are as follows:

Total per diem urine coproporphyrin (UCP)	18-99
Per diem coproporphyrin III	3-35
Per diem coproporphyrin I	10-90

From the Department of Medicine, University of Minnesota Hospital.

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‡The material for this part of the study was supplied through the courtesy of the local chapter of Alcoholics Anonymous, with the assistance of Dr. Charles Hewitt.

§We are indebted to Mr. Daniel O'Brien, Superintendent of Minneapolis Men's Workhouse, for making this study possible. It was carried out in the hospital section of the Workhouse.

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These were considered in more detail in paper I of this series.[4] It should be emphasized that this method relates only to preformed coproporphyrin and does not include the chromogen or precursor of coproporphyrin, the importance of which is discussed in separate communications.[5, 6]

Composite liver function studies, as discussed previously,[7] were also carried out in a majority of the cases. These usually included a fractional serum bilirubin, twenty-four hour urine urobilinogen, cephalin-cholesterol flocculation and thymol turbidity tests. In many instances, in addition, the bromsulfalein retention at forty-five minutes after injection of 5 mg. per kilogram of body weight, the prothrombin time, total and esterified serum cholesterol, and fractional serum proteins were determined.

RESULTS

It is evident from a review of the data in Tables I, II, and III that chronic alcoholism in general causes an elevation in the urine coproporphyrin excretion. This elevation occurs at the time of acute intoxication and the return toward normality following cessation of drinking varies in each case. Table I includes variations between the two extremes of a rapid return to normal and a slow return. Fig. 1 illustrates this variation.

The following case histories are those of the patients shown in Fig. 1.

CASE 39.—H. H., a 37-year-old man. For nine months this man was constantly inebriated to some degree. He drank an average of one quart of whisky a day, and he had not eaten a regular diet for at least eight to nine months. The patient showed some general improvement after five days of hospital care, during which period the porphyrin studies were made. Following release from the hospital, he resumed drinking and died five months later.

TABLE I. PERIOD OF ABSTINENCE AND TOTAL URINARY COPROPORPHYRIN VALUES (UCP)

PERIOD OF ABSTINENCE	URINE COPROPORPHYRIN (UCP) (GAMMA PER DAY)					NUMBER OF OBSERVATIONS*	PER CENT ABOVE NORMAL	AVERAGE VALUE
	0-100	1-200	2-300	3-400	400-			
Days								
1	6 (6)†	16 (14)	4 (4)	3 (3)	9 (9)	38	84	278
2	7 (7)	18 (18)	2 (2)		3 (3)	30	76	190
3	7 (7)	12 (12)	9 (5)	1 (1)	2 (2)	31	77	195
4	10 (10)	11 (11)	4 (2)		3 (3)	28	64	182
5	8 (8)	14 (14)	5 (2)			27	70	139
6	8 (8)	10 (10)	3 (1)	1 (1)	1 (1)	23	65	149
7	7 (7)	5 (5)	4 (4)	1 (1)		17	59	143
8	6 (6)	7 (7)	3 (1)	1 (1)		17	64	146
9	6 (4)	4 (2)	2 (2)			12	50	147
10	9 (3)	3 (3)		1 (1)		13	31	147
11-14	9 (7)	2 (2)				11	22	99
Weeks								
3	1 (1)	1 (1)				2		76
4	1 (1)					1		26
Months								
2, 3	15 (15)	5 (5)				20	33	67
4, 5	4 (4)	1 (1)				5	20	71
6-12	8 (8)	3 (3)				11	27	74
Years								
2-6	7 (7)					7	0	66

*Many cases were studied serially and therefore the same case may be represented at different periods. All of the observations for any one period, however, represent different individuals.

†The figure within parentheses indicates the number of persons and thus shows any duplication or "loading" of significance due to repeated observations on the same person.

CASE 40.—E. H., a 50-year-old man. This man had been drinking one pint of whisky daily for four months. Under treatment he recovered rapidly and returned to his job in seven days.

CASE 42.—J. G., a 47-year-old man. This chronic alcoholic had been drinking about one quart of whisky per day for seven months. He recovered physically but returned in a drunken state seven days after completion of this study.

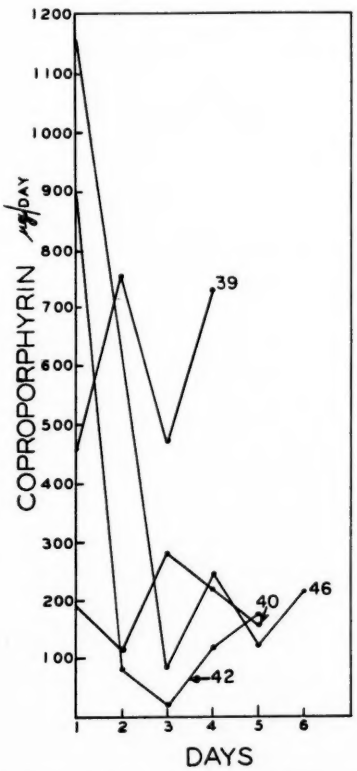

Fig. 1.—Serial data for total UCP in four alcoholic subjects studied from the first day of abstinence.

CASE 46.—A. M., a 30-year-old woman. This young woman had become a prostitute when 18 years old and a chronic alcoholic when 22 years old. She had been in a semi-stuporous condition from alcohol for a number of days at the time of admission. She had lost about 45 pounds in weight, was too weak to lift her arms on admission to the hospital, and remained in a critical condition for several weeks. She had hallucinations for the first two weeks. After this immediate period there was a constant gain in weight and in her general physical condition. She was discharged after two months of hospitalization showing no permanent neurological changes. Liver function studies on the second hospital day and on the twelfth day revealed:

DATE	SERUM BILIRUBIN 1′ (mg./100 c.c.)	SERUM BILIRUBIN TOTAL (mg./100 c.c.)	THYMOL TURBIDITY (units)	CEPHALIN-CHOLESTEROL (24 hr.)	CEPHALIN-CHOLESTEROL (48 hr.)	SERUM CHOLESTEROL (mg./100 c.c.)	SERUM CHOLESTEROL ESTERS (mg./100 c.c.)	(%)	24-HOUR URINE UROBILINOGEN
11/22/46	0.4	1.1	3	1+	2+	304	175 mg.	58	6.6 mg.
12/ 2/46	0.1	0.5	4	1+	1+	200	116 mg.	58	–

TABLE II. PERIOD OF ABSTINENCE AND URINE COPROPORPHYRIN III VALUES

PERIOD OF ABSTINENCE	URINE COPROPORPHYRIN III (GAMMA PER DAY)									NUMBER OF OBSERVATIONS*	PER CENT OF CASES OVER NORMAL	AVERAGE VALUE
	0-19	20-29	30-39	40-59	60-79	80-89	100-199	200-300	OVER 300			
Days												
1	2 (2)*	2 (2)	2 (2)	1 (1)	3 (3)	6 (6)	9 (9)		4 (4)	29 (29)	79	164
2	1 (1)		1 (1)	1 (1)	2 (2)	2 (2)	6 (6)		1 (1)	14 (14)	86	124
3				1 (1)	5 (5)	1 (1)	10 (10)	3 (3)	2 (2)	22 (22)	100	155
4				6 (6)	1 (1)	1 (1)	6 (6)	2 (2)	2 (2)	18 (18)	100	168
5			1 (1)	4 (4)	3 (3)	6 (6)	3 (3)	1 (1)		18 (18)	95	93
6		1 (1)	1 (1)	2 (2)	2 (2)	3 (3)	5 (5)		2 (2)	16 (16)	88	128
7			2 (1)			2 (1)	7 (7)	2 (2)	1 (1)	14 (13)	87	143
Weeks												
2	2 (2)		3 (2)	11 (6)	5 (4)	2 (1)	13 (8)	4 (3)	1 (1)	41 (27)	88	101
3			1 (1)		2 (2)	2 (2)	2 (1)			7 (6)	85	90
4					1 (1)					1 (1)	100	73
Months												
2-4		2 (2)	4 (3)	8 (4)	9 (5)	4 (3)	5 (5)	2 (1)		34 (23)	83	77
5-7		1 (1)		1 (1)	2 (2)		1 (1)			5 (5)	80	69
8-12	1 (1)		1 (1)	2 (2)	1 (1)					5 (5)	60	46
Years												
2-4	1 (1)		1 (1)	1 (1)						3 (3)	33	21

*See footnotes to Table I.

TABLE III. PERIOD OF ABSTINENCE AND URINE COPROPORPHYRIN I VALUES

PERIOD OF ABSTINENCE	URINE COPROPORPHYRIN I (GAMMA PER DAY)									NUMBER OF CASES*	PER CENT OF CASES OVER NORMAL	AVERAGE VALUE
	0-19	20-29	30-39	40-59	60-79	80-99	100-199	200-300	OVER 300			
Days												
1	6 (6)*	5 (5)	1 (1)	2 (2)	7 (7)	4 (4)	2 (2)	1 (1)	1 (1)	29 (29)	14	70
2	4 (4)	2 (2)	3 (3)	2 (2)			2 (2)	1 (1)		14 (14)	21	59
3	8 (8)	4 (4)	4 (4)	3 (3)	1 (1)		1 (1)	1 (1)		22 (22)	9	34
4	6 (6)	5 (5)	1 (1)	1 (1)	1 (1)	2 (2)	1 (1)			18 (18)	11	49
5	10 (10)	2 (2)	2 (2)	3 (3)	1 (1)					18 (18)	0	23
6	6 (6)	2 (2)	3 (3)	5 (5)						16 (16)	0	29
7	7 (7)	2 (2)	4 (2)	1 (1)	1 (1)					15 (13)	0	20
Weeks												
2	28 (15)	3 (2)	3 (3)	5 (5)	1 (1)		1 (1)			41 (27)	2	19
3	6 (5)	1 (1)								7 (6)	0	14
4	1 (1)									1 (1)	0	14
Months												
2-4	20 (13)	7 (5)	4 (2)	2 (2)		1 (1)				34 (23)	0	20
5-7	3 (3)	2 (2)								5 (5)	0	17
8-12	4 (4)	1 (1)								5 (5)	0	14
Years												
2-6	2 (2)			1 (1)						3 (3)	0	22

*See footnotes to Table I.

Although not included in Fig. 1, the case of W. K. is described for its many interesting features. This 50-year-old white man had been an uncontrolled alcoholic for three years when first seen on the psychiatric service at Minneapolis General Hospital on May 16, 1947. He had been drinking one pint of whisky a day for one month and eating very little. He was weak, shaky, and suffering from auditory and visual hallucinations. He was readmitted in one month in a similar condition and discharged in July. This man continued to appear at intervals in delirium tremens after prolonged drinking episodes during the next two years. The final study was done in the city workhouse in January, 1949. At this time he had his tenth episode of delirium tremens which lasted for three days. During the three-day period he had for the first time frequent generalized convulsions. This individual has never shown any clinical signs of liver disease. On each admission to the hospital or the workhouse his manner and appearance was that of an alcoholic in acute distress, but he would again appear normal after a few days. He suffered no apparent permanent organic or functional changes in the central nervous system. The liver function tests have always remained normal; the urinary coproporphyrin values would be high following a period of alcoholism but during his only long period of total abstinence (a ninety-day workhouse sentence) they became normal. A liver biopsy was done and the morphology was completely normal, although he had been drinking to within a few weeks of the biopsy and had recently recovered from delirium tremens.

The laboratory data on a typical admission are shown in Table IV.

TABLE IV

DATE	3/28	3/30	4/1	4/3	4/5	5/11	5/23
Urine coproporphyrin (UCP) in γ per 24 hr.	520	468	618	420	260	78	68
Per cent isomer III	90	89	95	86	75	50	50
γ of type III isomer per 24 hr.	468	417	587	361	195	39	34
γ of type I isomer per 24 hr.	52	51	31	59	65	39	34
Serum bilirubin l′ (prompt direct) in mg. per 100 c.c.			0.07		0.15		
Total serum bilirubin (prompt direct) in mg. per 100 c.c.			0.64		0.88		
Thymol turbidity (units)			1.1		1.4		
Cephalin cholesterol			0		0		
Total serum cholesterol in mg. per 100 c.c.			170		214		
Per cent esterified cholesterol			73		71		
Alkaline phosphatase (Bodansky units)					1.6		

It was noted during the study that a few chronic alcoholics had normal or only slightly elevated UCP values during recovery from impending delirium tremens. This was in contrast to the many that had elevated values. Two possible explanations were considered for this disparity. (1) Some individuals may be able to drink large amounts of alcohol over a prolonged period with no change in their porphyrin metabolism. (2) The men studied were in the workhouse or Minneapolis General Hospital (sent in from the city jail, workhouse, or their homes) and had often had no alcohol to drink for twenty-four to seventy-two hours. The peak of coproporphyrin excretion thus might have been passed when they were admitted. These patients were sent to the hospital units because of impending or actual delirium tremens and this usually develops three to five days after the cessation of drinking. The necessity of determining the effect of drunkenness on coproporphyrin metabolism under controlled experimental conditions was evident.

The problem was studied in the following manner. (a) Chronic alcoholics serving workhouse sentences volunteered to take alcohol as directed. (b) The

men chosen were all serving terms of such length that they had consumed no alcohol for at least two weeks preceding administration of the alcohol. (c) 95 per cent ethyl alcohol was used mixed with Coca Cola or sweetened water. (d) The men were allowed to mix the alcohol as desired and the drinking was continued until the person was definitely intoxicated or had consumed at least 250 ml., usually in a three-hour period. (3) Urine was collected for a period preceding the test, during the time of drinking, and for some days following.

The first person studied became intoxicated after taking 250 ml. of 95 per cent alcohol in a three-hour period. There was a rise in the UCP, due to increased amounts of the type III isomer. (Case 1, Table V.)

The second man studied had a remarkable tolerance for alcohol and consumed the same amount in the same period of time with little, if any, visible effect. There was no rise in his coproporphyrin excretion. After one week the same individual was given alcohol again and continued to drink until he became unconscious. However, there was again little change in his behavior at the time he had drunk 250 ml.; at 290 ml. he became quite inebriated; at 330 ml., he became unconscious. He was unconscious for six hours and remained inebriated for over twenty-four hours. With this amount of alcohol there was a definite,

TABLE V. ACUTE ALCOHOLIC INTOXICATION AND URINE COPROPORPHYRIN VALUES

CASE 1

TIME (DAYS)	1	2	3	4	5*	6	7	8	9	10
UCP	135	127	106	56	228	101	81			107
III/day	123	116	50	51	205	91	78			103
I/day	12	11	56	5	23	10	3			4

*250 ml. 95 per cent alcohol; individual severely intoxicated

CASE 2

TIME (DAYS)	1	2	3*	4	5	6	7	8	9	10†	11	12	13	14	15
UCP	71	104	74	41	104	103	84	104	70	168	184	249	100	52	93
III/day	32	47			47	47	42	54	36	120	164	228			
I/day	39	57			57	56	42	50	34	26	20	20			

*200 ml. 95 per cent alcohol; not inebriated
†330 ml. 95 per cent alcohol; Individual unconscious for six hours and inebriated to a moderate degree for the following twelve hours

CASE 3

TIME (DAYS)	1	2	3	4	5*	6	7	8
UCP	138	108	84	93	165	175	186	87
III/day	98	77	72	80	140	152	160	78
I/day	40	31	12	13	21	23	26	9

*250 ml. 95 per cent alcohol; individual mildly intoxicated

CASE 4

TIME (DAYS)	1	2	3	4	5	6	7	8	9*	10	11	12	13	14	15
UCP	139	93	80	98	115	80	55	61	102	149	222	199	307	102	144
III/day	117	69	73		101	58	40	54	90	95	189	159	279	90	134
I/day	22	24	7		14	22	15	7	12	54	33	30	21	12	10

*190 ml. 95 per cent alcohol; individual unconscious for three hours and inebriated for many hours following return of consciousness

CASE 5

TIME (DAYS)	1	2	3	4*	5	6	7	8
UCP	67	66	76	112	134	87	80	63
III/day	58	57	66	96	103	72		55
I/day	9	9	10	16	31	15		8

*200 ml. 95 per cent alcohol; individual only slightly inebriated

rapid, marked rise in the UCP value and this was due to the type III isomer (Case 2, Table V). In repeated studies with this individual it was found that he did not become inebriated until he had taken at least 280 ml. of alcohol in a 2.5 to 3.0 hour interval and that he would become unconscious at about 330 ml. if that amount was taken in a three-hour period.

DISCUSSION

The literature contains relatively little information as to the effect of alcohol on coproporphyrin metabolism. A small amount of data has been recorded relating to the increases of the total urinary coproporphyrin as a result of alcohol ingestion. Thus Franke and Fikentscher[8] and Franke[9] observed distinct increases following relatively small amounts of beer or cognac. They believed that this was related simply to liver injury and that the urinary coproporphyrin was a very delicate test of liver functional disturbance. Brugsch and Keys[10] also noted increased coproporphyrinuria in acute experiments with alcohol in human beings. In addition they observed increases in individuals with chronic alcoholism and they were the first to report any data on the isomers. They isolated the type I isomer from two cases and the type III isomer from one. This indicates quite the opposite from what has been found in the present study, i.e., a rather consistent and often marked increase of the type III isomer. It will be noted in Table I, however, that there were certain instances in which the type I isomer was preponderant, and since the ester of type I crystallizes much more readily than that of type III it is not unlikely that if isolation had been carried out in these instances the type I isomer would have been obtained. It seems probable that Brugsch and Keys were working with similar exceptional cases. Watson[11] later isolated coproporphyrin III from the urine of a patient with alcoholic pellagra. Dobriner and co-workers[12] found an increased urinary coproporphyrin in a case of alcoholic pellagra and identified both I and III isomers. The increases in both of these studies may be assumed to be related to the alcoholism since the urinary coproporphyrin was not found to be increased in endemic pellagra.[13-16]

It is evident from the present data that alcohol commonly causes excessive excretion of the type III isomer. Nevertheless, as noted in Table I, there are occasional unexplained exceptions, and it is further seen in Table III that a minority of chronic alcoholics excrete increased amounts of type I isomer during the relatively early period of abstinence. This may be due to functional impairment of the liver.

The mechanism responsible for the preponderant increase of type III coproporphyrin after alcohol is unknown. Several possibilities deserve comment. It appears from the present acute experiments that there is correlation with the symptoms of the effect of alcohol on the central nervous system. The earlier reports of Franke and Fikentscher[8] and Brugsch and Keys[10] do not permit accurate comparisons in this regard because exact time relationships and degree of intoxication were not stated. The results of the former study indicate significant increases of urinary porphyrin with amounts of alcohol well below the in-

toxicating level. Brugsch and Keys gave amounts ranging from 300 to 450 c.c. of whiskey during one day, but the exact period of drinking was not stated. These are smaller than were required to produce significant increases in the present study, but they were dealing with "normal" men while the present subjects were hardened alcoholics in the truest sense. The increases shown in Brugsch and Keys' paper are of questionable significance in that but one determination exceeded the normal and the fluctuation of UCP from day to day is well known. The increases reported by Franke and Fikentscher were also of relatively minor degree, and within the normal range. It is evident that further and more detailed studies in normal as compared with alcoholic subjects are needed.

There is a question as to whether the excesses of type III coproporphyrin in the urine are possibly derived from the small amounts which are known to be present normally in the central nervous system.[17, 18, 19] At least one objection to this concept is that the greatest degree of increase often did not appear until the second or third day after the alcohol, whereas if the increase simply represented coproporphyrin liberated from the nervous system it might have been expected to appear much earlier. One must reckon with the possibility, however, that overproduction of coproporphyrin in the nervous system is not made manifest in the urine until some time after the effect of the alcohol. The central nervous system origin cannot be excluded when one considers that there is commonly an increase of type III isomer in the urine of similar magnitude in acute poliomyelitis[20] while in infectious hepatitis the excess is due to the type I isomer.[21]

In the preceding paper of this series[1] it was shown that the increased excretion of the type III isomer in cases of alcoholic cirrhosis is commonly at a much higher level and continues for longer periods of time than in simple alcoholism as in the present series. Nevertheless, it is evident in Fig. 1 that there is considerable individual variation in the rate of decline of the UCP after the beginning of abstinence. Furthermore a comparison of Tables I and II reveals clearly that in many instances the isomer ratio remains disturbed, in favor of a preponderance of type III when the total UCP has fallen within the normal range. This is best seen by contrasting the data in the next to last column in the two tables, especially that related to months after beginning abstinence. This, of course, implies that a disturbance in the liver might be responsible for the excessive type III isomer excretion. The possibility was discussed that the excesses were representative of a disturbance in liver cell metabolism. Many substances which inhibit enzyme systems such as heavy metals and various chemicals are also associated with excessive type III coproporphyrin excretion in the urine, and it is probable that the coproporphyrins are intimately related to cellular metabolism, more particularly in the bone marrow, liver, and nervous system. Thus far it has not been possible to determine where the increases noted in the alcoholic subjects originate. The bone marrow cannot be excluded. In another study which will be reported separately, the urinary and erythrocyte porphyrins (including coproporphyrin) have been determined in patients with porphyria receiving alcohol in acute experiments as well as in a small group of chronic alcoholics. Although significant and often considerable increases of urinary

porphyrin have been observed in both groups, the erythrocyte porphyrins did not show any definite changes. This, of course, does not exclude the possibility that the excessive UCP is formed in the bone marrow. Nevertheless, it would appear to point to some other site as being more likely.

SUMMARY AND CONCLUSIONS

In a series of chronic alcoholic subjects studied at various intervals after last drinking, significant and often marked increases of urinary coproporphyrin were observed. In general, the longer the interval after drinking the lesser was the tendency of the UCP to increase, but marked variations were observed in the rate at which the values returned to normal after cessation of drinking.

In the main the increased UCP was due to increase of the type III isomer, and in a number of instances this increase occurred without appreciable evidence of liver functional impairment. In some cases there was also a moderate increase of type I isomer.

In acute experiments in a group of five chronic alcoholic subjects the effect of relatively large amounts of alcohol given in a short space of time was observed. Significant and often considerable increases of UCP were noted, the largest amounts often not appearing until from two to four days after the intoxication. This would appear to indicate a new formation of porphyrin as the result of the alcohol, rather than a release of preformed porphyrin. The site of formation has not been determined.

REFERENCES

1. Watson, C. J., Sutherland, D., and Hawkinson, V.: Studies of Coproporphyrin. V. The Isomer Distribution and Per Diem Excretion of the Urinary Coproporphyrin in Cases of Cirrhosis of the Liver, J. LAB. & CLIN. MED. **37**: 8, 1951.
2. Watson, C. J., and Larson, E. M.: The Urinary Coproporphyrins in Health and Disease, Physiol. Rev. **27**: 478, 1947.
3. Schwartz, S., Hawkinson, V., Cohen, S., and Watson, C. J.: A Micromethod for the Quantitative Determination of the Urinary Coproporphyrin Isomers (I and III), J. Biol. Chem. **168**: 133, 1947.
4. Watson, C. J., Hawkinson, V., Schwartz, S., and Sutherland, D.: Studies of Coproporphyrin. I. The Per Diem Excretion and Isomer Distribution of Coproporphyrin in Norman Human Urine, J. Clin. Investigation. **28**: 447, 1949.
5. Watson, C. J., Pimenta de Mello, R., Schwartz, S., Hawkinson, V., and Bossenmeier, I.: Porphyrin Chromogens in Urine and Blood. To be published.
6. Schwartz, S., Zieve, L., and Watson, C. J.: An Improved Method for the Determination of Urinary Coproporphyrin and an Evaluation of Pertinent Factors Involved in the Analysis. To be published.
7. Watson, C. J., and Hoffbauer, F. W.: Liver Function in Hepatitis, Ann. Int. Med. **26**: 813, 1947.
8. Franke, K., and Fikentscher, R.: Die Bedeutung der quantitativen Porphyrinbestimmung mit der Lumineszenzmessung für die Prüfung der Leberfunktion und für Ernährungsfragen, München. med. Wchnschr. **82**: 171, 1935.
9. Franke, K.: Klinische und lebendmikroskopiscke Untersuchungen der gestörten Leberfunktion. II. Untersuchungen über beginnende Leberschädigungen unter besonderer Berücksichtigung der Porphyrinausscheidung im Urin, Ztschr. f. klin. Med. **130**: 222, 1936.
10. Brugsch, J., and Keys, A.: Toxic Porphyrinuria Following Chronic Alcoholism, Proc. Staff Meet., Mayo Clin. **12**: 609, 1937.
11. Watson, C. J.: The Urinary Pigments in Four Cases of Alcoholic Pellagra, Proc. Soc. Exper. Biol. & Med. **39**: 514, 1938.
12. Dobriner, K., Strain, W. H., and Localio, S. A.: The Excretion of Porphyrin in Pellagra, Proc. Soc. Exper. Biol. & Med. **38**: 748, 1938.

13. Watson, C. J.: Further Observations on the Red Pigments of Pellagra Urines, Proc. Soc. Exper. Biol. & Med. **41**: 591, 1939.
14. Watson, C. J., and Layne, J. A.: Studies of Urinary Pigments in Pellagra and Other Pathological States. I. Clinical observations, Ann. Int. Med. **19**: 183, 1943.
15. (a) Meiklejohn, A. P., and Kark, R.: Pigment Excretion in Pellagra, New England J. Med. **221**: 519, 1939.
    (b) Kark, R., and Meiklejohn, A. P.: Pellagra and Porphyrinuria, Am. J. M. Sc. **201**: 380, 1941.
16. Rimington, C., and Leitner, Z. A.: Urinary Excretion of Coproporphyrin in Non-alcoholic Pellagra, Lancet **2**: 494, 1945.
17. Klüver, H.: On Naturally Occurring Porphyrins in the Central Nervous System, Science **99**: 482, 1944.
18. Klüver, H.: Porphyrins, the Nervous System, and Behavior, J. Psychol. **17**: 209, 1944.
19. Ju-Hwa Chu, E., and Watson, C. J.: An Experimental Study of the Cerebral Coproporphyrin in Rabbits, Proc. Soc. Exper. Biol. & Med. **66**: 569, 1950.
20. Watson, C. J., Schulze, W., Hawkinson, V., and Baker, A. B.: Coproporphyrinuria (Type III) in Acute Poliomyelitis, Proc. Soc. Exper. Biol. Med. **64**: 73, 1947.
21. Watson, C. J., Hawkinson, V., Capps, R. B., and Rappaport, E. M.: Studies of Coproporphyrin. IV. The Per Diem Excretion and Isomer Distribution in the Urine in Infectious Hepatitis, Infectious Mononucleosis, and Mechanical Jaundice, J. Clin. Investigation **28**: 621, 1949.

STUDIES OF LIVER FUNCTION TESTS

II. Derivation of a Correction Allowing Use of the Bromsulfalein Test in Jaundiced Patients

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IT IS generally assumed that the bromsulfalein test is not interpretable in the presence of moderate or severe grades of jaundice presumably because bromsulfalein like bilirubin is returned to the blood stream in the presence of regurgitation. So much emphasis has been placed on the undesirability of performing this test in the presence of jaundice that its predictive value in the less severely jaundiced patient has been ignored in many instances.

The bromsulfalein test is one of the best tests of hepatocellular dysfunction. Its use in measuring the degree of such dysfunction is no less important in the jaundiced than in the nonjaundiced patient, particularly in view of evidence to be presented in a later paper of this series accentuating its value as a measure of a basic function of the liver. In addition it is one of the simplest tests to perform.

In the jaundiced patient the obtained bromsulfalein value may be thought of as consisting of two parts, bromsulfalein retained in the serum and bromsulfalein returned to the serum because of regurgitation of some type. If one could segregate these two portions and calculate a score for the serum bromsulfalein due to the regurgitation mechanism, one should be able to interpret the test in the presence of jaundice. Such a score may be derived from a plot of the relationship of bromsulfalein retention and serum bilirubin concentration in patients having liver disease or extrahepatic obstructive jaundice.

METHODS AND MATERIALS

The case material consisted of ninety-four consecutive patients with liver disease or extrahepatic obstructive jaundice. A battery of liver function tests was given to each patient. Included were the combined intravenous bromsulfalein-hippuric acid-galactose test, one-minute (1'Bi.) and total bilirubin (Tot.Bi.), thymol turbidity (T.T.), zinc sulfate turbidity (Z.T.), cholesterol ester (CE) per cent, twenty-four hour urine urobilinogen (U.U.), twenty-four hour urine coproporphyrin (UCP), cephalin-cholesterol flocculation (CCF), and colloidal red (CR) tests. The combined test is one in which bromsulfalein (BSP), sodium benzoate, and

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galactose (Gal.) are injected simultaneously and specimens collected at appropriate times, forty-five minutes in the case of bromsulfalein and sixty minutes in the case of galactose and hippuric (Hipp.) acid.[1] The limit of normal for the galactose test is 40 mg. per cent. The one-minute bilirubin determination was used routinely because of its independent diagnostic value. This was first indicated by Watson[2] and will be demonstrated further in a subsequent paper. A new simplified method involving a determination on 5 c.c. of urine preserved with sodium carbonate was used in the coproporphyrin determination.[3] Values up to 300 μg are normal. Twenty-four and forty-eight hour readings were recorded for the colloidal red test, by analogy with the CCF, even though Ducci[4] recommended the first reading only.

A graph was made relating the bromsulfalein to the one-minute and total bilirubin values. The region of significant association of the bromsulfalein and bilirubin scores was delineated, and an equation summarizing this relationship was derived. With this equation an adjustment was calculated which allows one to determine the expected value of the bromsulfalein corresponding to some arbitrary bilirubin value below the region of significant association. The basis for this correction involves an elementary statistical concept which is presented in the following section.

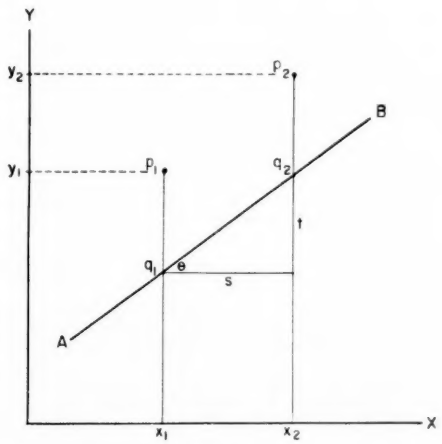

Fig. 1.

STATISTICAL CONCEPT

Suppose we are dealing with two variables x and y which are related by a line AB as illustrated in Fig. 1. Suppose further that corresponding to a score x_2 on the x-axis there is a distance y_2 on the y-axis giving the point p_2; and corresponding to x_1 is y_1 giving the point p_1. We wish to calculate y_1 knowing y_2 and the equation of the line AB. Of the total distance y_2 up to the point p_2, the portion x_2q_2 could have been predicted from the equation of the line AB and the rest, q_2p_2, could not, being independent of the line. Assuming the portion independent of the line remains unchanged, we would expect that when dropping from x_2 to x_1, y_2 would decrease by an amount equivalent to the difference $x_2q_2 - x_1q_1$, represented by t in the figure. Now $\tan \theta = \frac{t}{s}$ where θ is the angle made by the line AB with any line parallel to the x-axis. If AB represents a regression line, its regression coefficient, b, is the slope of the line, i.e. $b = \tan \theta$, and $t = bs = b(x_2 - x_1)$. Therefore $b(x_2 - x_1)$ is the amount to be subtracted from y_2 to get the value y_1 corresponding to x_1. If p_2 were below the line instead of above it, the adjustment would be the same except that q_2p_2 would be negative instead of positive.

In the application below, x_2 is the obtained bilirubin value, y_2 the obtained bromsulfalein value, x_1 the highest bilirubin value below the region of significant association of the two variables, and y_1 the expected bromsulfalein value corresponding to x_1, given the obtained regression line.

RESULTS

Relationship of Bromsulfalein to One-Minute Bilirubin.—In Fig. 2 a plot of the bromsulfalein against the logarithm of the one-minute bilirubin value is represented by the large dots. Upon examination it is apparent that one can divide this graph into two portions with the dividing line approximately at a one-minute value of 1.0 mg. per cent. The portion below this point shows no

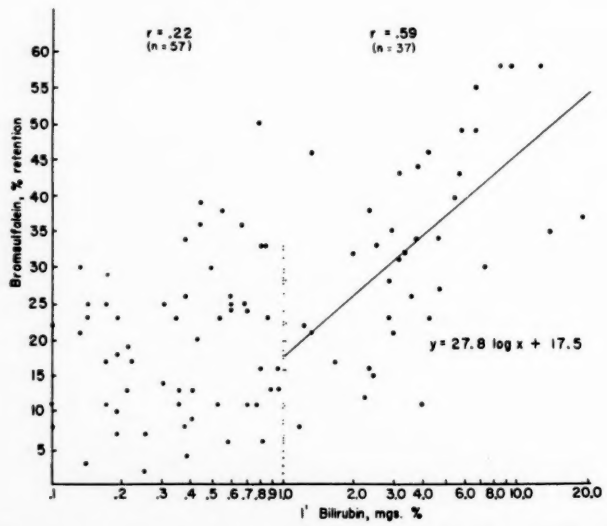

Fig. 2.—Relationship of bromsulfalein and one-minute bilirubin. The column of small dots along the ordinate at a one-minute bilirubin of 1.0 mg. per cent represents the corrected oromsulfalein values corresponding to the points scattered about the regression line.

correlation between the bromsulfalein and bilirubin scores. The coefficient is 0.22, which is insignificantly different from zero. Above this point the variables correlate significantly, the coefficient being 0.59. Though it is based on a relatively small sample of thirty-seven cases, this coefficient probably closely approximates the true correlation. The reliability of the bromsulfalein and bilirubin tests in the region studied is nearly perfect so the coefficient corrected for attenuation is practically unchanged. The regression of bromsulfalein on one-minute bilirubin in this region is represented by the line, $y = 27.8 \log x + 17.5$, which is drawn in on the chart. This line gives us the average value of the bromsulfalein corresponding to any given value of the bilirubin. The deviations of the points from the line in the vertical direction represent the portions of the bromsulfalein scores that are not accounted for by the bilirubin values. Any one of the points corresponds to the point p_2 in Fig. 1. The cases with the most severe liver dysfunction tend to fall above the line, and those with disproportionate jaundice fall below the line. The extreme cases of pure obstruction without

liver dysfunction, which have stabilized, would be expected to fall farthest below the line and parallel to it.

We may use this equation, employing the principle discussed previously, to adjust the bromsulfalein score so as to eliminate the portion associated with the bilirubin elevation, i.e., the portion due to regurgitation. What is left presumably represents retention. Assuming the deviations from the line remain unchanged, we may calculate by means of the regression line the amount that any bromsulfalein value (corresponds to y_2) would decrease on the average when the corresponding bilirubin value (corresponds to x_2) is adjusted down

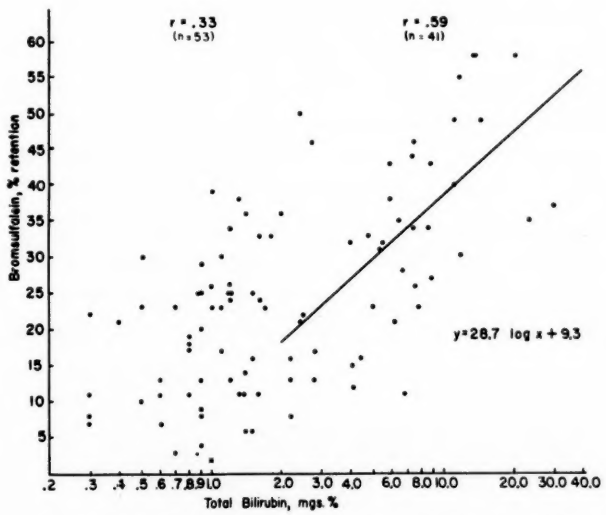

Fig. 3.—Relationship of bromsulfalein and total bilirubin.

to a bilirubin of 1.0 mg. per cent (corresponds to x_1). The latter point represents the upper limit of the region where there is no correlation between the two variables. The amount of decrease is given by 27.8 (log x – log 1) or 27.8 log x [corresponds to $b(x_2 - x_1)$] where x is the bilirubin value we start with. The bromsulfalein values for the points about the regression line after applying this correction are shown as small dots along the ordinate at a bilirubin of 1.0 mg. per cent. The distribution of these values after adjustment parallels the plot below this level, the region where jaundice does not appreciably influence the bromsulfalein value.

Relationship of Bromsulfalein to Total Bilirubin.—Fig. 3 presents the relationship of the bromsulfalein to the logarithm of the total bilirubin for the same patients. The graph is similar to Fig. 2. In this instance the dividing line is approximately a total bilirubin of 2.0 mg. per cent. Below this point the correlation of bromsulfalein and total bilirubin is 0.33, a value significantly different from zero, though indicating but slight association of the two variables in this region. Above this point the correlation is 0.59. The regression of brom-

sulfalein on total bilirubin in this region is represented by the line, $y = 28.7 \log x + 9.3$, which is drawn in on the chart. It will be noted that the correlation of 0.59 is identical to that for bromsulfalein and log one-minute bilirubin. This results from the fact that in nonhemolytic jaundice the total and one-minute bilirubin are nearly perfectly correlated ($r = 0.99$) above the one-minute level of 1.0 mg. per cent.

As in the case of Fig. 2, we could use the regression equation to adjust the bromsulfalein on the basis of its association with the total bilirubin. The result would be similar; however, the total bilirubin has the slight disadvantage of significant association with the bromsulfalein below the dividing line.

Practical Use of the Formula.—To expedite the use of the correction formula we have constructed a table giving the amount to be subtracted from the bromsulfalein corresponding to various levels of the one-minute bilirubin from 1.0 mg. per cent to 25 mg. per cent (Table I). We have also constructed a nomograph (Fig. 4) which allows the corrected value to be read directly given the initial bromsulfalein and one-minute bilirubin values. A ruler is laid across the graph connecting the obtained bromsulfalein (BSP) and one-minute bilirubin scores, and the desired value read off where it intersects the column headed Corrected BSP. Two examples illustrating the use of the table and graph follow.

TABLE I. TABLE OF VALUES TO BE SUBTRACTED FROM BROMSULFALEIN TEST RESULT, CORRESPONDING TO VARIOUS LEVELS OF ONE-MINUTE BILIRUBIN

ONE-MINUTE BILIRUBIN	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1	0	1	2	3	4	5	6	6	7	8
2	8	9	10	10	11	11	12	12	12	13
3	13	14	14	14	15	15	15	16	16	16
4	17	17	17	18	18	18	18	19	19	19
5	19	20	20	20	20	21	21	21	21	21
6	22	22	22	22	22	23	23	23	23	23
7	23	24	24	24	24	24	24	25	25	25
8	25	25	25	26	26	26	26	26	26	26
9	27	27	27	27	27	27	27	27	28	28
10	28	28	28	28	28	28	28	29	29	29
11	29	29	29	29	29	29	30	30	30	30
12	30	30	30	30	30	30	31	31	31	31
13	31	31	31	31	31	31	31	32	32	32
14	32	32	32	32	32	32	32	32	33	33
15	33	33	33	33	33	33	33	33	33	33
16	33	34	34	34	34	34	34	34	34	34
17	34	34	34	34	34	35	35	35	35	35
18	35	35	35	35	35	35	35	35	35	35
19	36	36	36	36	36	36	36	36	36	36
20	36	36	36	36	36	36	37	37	37	37
21	37	37	37	37	37	37	37	37	37	37
22	37	37	37	37	38	38	38	38	38	38
23	38	38	38	38	38	38	38	38	38	38
24	38	38	38	39	39	39	39	39	39	39

NOMOGRAPH FOR COMPUTING BSP CORRECTED
FOR ELEVATION DUE TO JAUNDICE
(FORMULA, $Y_c = Y - 27.8 \log X$)

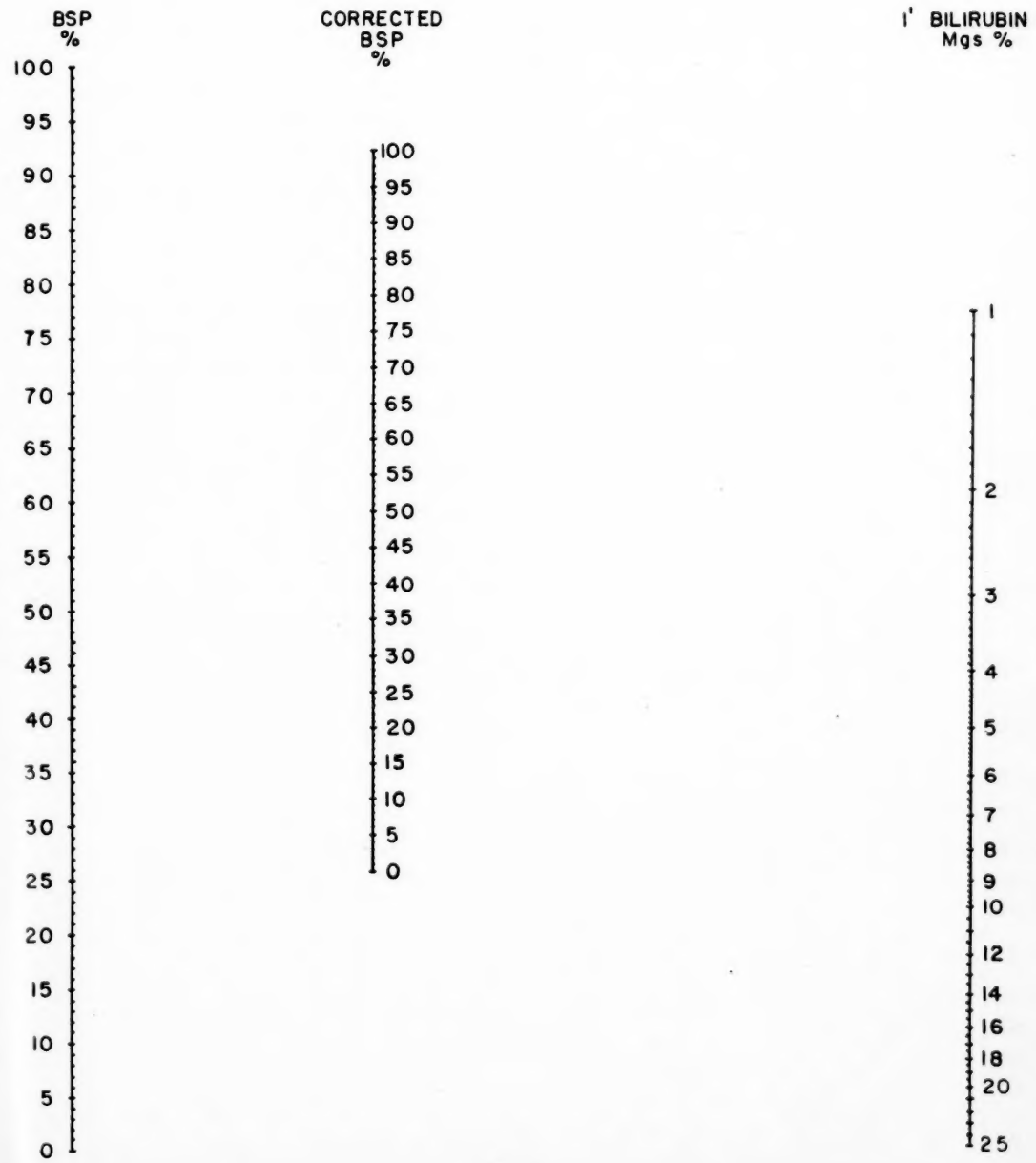

Fig. 4.

Example 1: Given BSP of 37 per cent, one-minute bilirubin of 18.9 mg. per cent. In Table I the figure 35 is found corresponding to the one-minute bilirubin value of 18.9. This is subtracted from 37 giving 2 as the corrected bromsulfalein. In Fig. 4 a ruler connecting 37 in the BSP column with 18.9 in the one-minute bilirubin column intersects the corrected BSP column at 2.

Example 2: Given BSP of 55 per cent, one-minute bilirubin of 7.0 mg. per cent. In Table I the figure 23 is found corresponding to the one-minute bilirubin value of 7.0 This is subtracted from 55 giving 32 as the corrected brom-

TABLE II. LIVER FUNCTION TEST RESULTS IN PATIENTS

CASE	AGE	WT.	1′ BI.	TOT. BI.	BSP	CORRECTED BSP	T.T.	Z.T.	% CE
Z 92	32	123	3.88	6.8	11	−5	6.9	18	70
Z 90	53	136	18.95	30.0	37	2	3.9	11	34
Z 103	20	141	2.21	4.1	12	2	9.2	15	69
Z 62	50	140	13.66	23.6	35	4	6.9	22	17
Z 106	53	136	2.39	4.1	15	5	14.0	14	70
Z 1	41	162	7.18	12.0	30	6	1.7	2	26
Z 8	44	142	1.14	2.2	8	6	2.7	10	71
Z 69	50	108	4.18	7.9	23	6	17.0	22	64
Z 70	51	155	2.30	4.4	16	6	22.5	25	69
Z 15III	34	122	2.92	6.2	21	8	11.3	26	64
Z 99	61	146	4.61	8.9	27	9	33.7	30	60
Z 5	51	152	1.65	2.8	17	11	3.4	12	29
Z 46	55	178	3.49	7.6	26	11	2.2	9	69
Z 100	27	155	2.82	5.0	23	11	2.9	7	70
Z 20	39	129	4.59	8.7	34	16	5.5	7	41
Z 36	39	177	2.82	6.7	28	16	1.7	4	39
Z 35	42	133	1.31	2.4	21	18	4.7	10	56
Z 75	30	138	3.65	7.5	34	18	30.2	26	57
Z 94	37	180	3.27	5.5	32	18	11.6	7	44
Z 98	36	132	3.07	5.3	31	18	8.5	7	55
Z 17	62	155	5.39	11.2	40	20	15.0	7	29
Z 82	30	151	1.21	2.5	22	20	9.6	14	76
Z 24	42	137	2.46	4.8	33	22	11.7	9	43
Z 47	40	167	2.92	6.5	35	22	10.7	21	63
Z 95	53	124	5.64	8.8	43	22	7.2	7	58
Z 80	31	180	1.95	4.0	32	24	7.2	15	59
Z 57	53	124	6.67	14.5	49	26	11.4	5	77
Z 39	21	117	5.72	11.2	49	28	5.7	10	43
Z 40	21	148	12.30	20.5	58	28	7.3	9	17
Z 42	56	107	2.30	5.9	38	28	14.7	29	47
Z 66	61	174	3.71	7.4	44	28	2.7	9	40
Z 7	52	195	4.10	7.5	46	29	8.8	20	46
Z 55	51	127	3.10	5.9	43	29	9.8	12	31
Z 44	63	330	9.15	13.5	58	31	16.4	34	37
Z 96	56	135	6.51	11.8	55	32	8.9	8	59
Z 105	72	166	8.36	13.6	58	32	3.5	3	28
Z 87	24	127	1.27	2.7	46	43	6.8	13	59
Mean	44	150	4.67	8.5	33	18	9.7	14	51
Range	20-72	107-330	1.14-18.95	2.2-30.0	8-58	0-43	1.7-33.7	2-34	17-77

1′ Bi., One-minute bilirubin, mg. %.
Tot. Bi., Total bilirubin, mg. %.
BSP, Bromsulfalein retention, 45′, %.
T.T., Thymol turbidity, $CuSO_4$ units.
Z.T., $ZnSO_4$ turbidity, $BaSO_4$ units.
% CE, Per cent cholesterol esters.

sulfalein. In Fig. 4 a ruler connecting 55 in the BSP column with 7.0 in the one-minute bilirubin column intersects the corrected BSP column at 31.8, which we round off to give 32.

DISCUSSION

Validity of the Correction.—The validity of this correction will be judged by the extent to which the adjusted bromsulfalein values correlate with other evidences of dysfunction, generally other function tests. It should be recognized in advance, however, that in a mixed group of disease states such comparisons

WITH ONE-MINUTE BILIRUBIN GREATER THAN 1.0 MG. PER CENT

GAL.	HIPP.	U.U.	5 C.C. UCP	CCF 24, 48	CR 24, 48	CLINICAL DIAGNOSIS
10	1.17	10.8	314	3, 3	1, 2	Seminoma w. liver met., after trt.
46	0.50	1.0	714	3, 3	0, 0	C. D. (common duct) obstr., met. from Ca stom.
25	1.12	4.2	320	1, 1	4, 5	Serum hepatitis, mild
51	0.19	11.2	553	4, 4	0, 0	Serum hepatitis, mod.
13	0.48	2.3	226	2, 2	4, 4	Serum hepatitis, mild
5	0.72	20.7	356	0, 0	0, 0	C. D. obstr., bil. cirrhosis
15	0.97	1.3	120	0, 2	3, 4	Serum hepatitis, mild
66	0.40	7.3	442	0, 1	2, 3	Serum hepatitis, mild
27	0.88	11.3	568	3, 3	4, 4	Serum hepatitis, mild
87	0.51	15.2	309	4, 4	4, 4	Portal cirrhosis, mod.
29	0.38	21.2	515	4, 4	5, 5	Serum hepatitis, mod.
50	0.95	12.4	442	0, 1	1, 1	Portal cirrhosis, sev.
84	0.91	9.1	420	0, 0	0, 0	Serum hepatitis, mild
30	0.77	7.2	271	2, 2	0, 0	Serum hepatitis, mod.
85	0.88	15.2	330	0, 1	2, 3	Serum hepatitis, mild
34	0.51	1.0	790	0, 0	0, 0	C. D. obstr., Ca pancreas, bil. cirrhosis
67	0.66	18.4	801	4, 4	4, 4	Portal cirrhosis, sev.
63	0.81	30.2	607	4, 4	0, 0	Portal cirrhosis, sev.
41	0.53	71.0	829	3, 3	0, 0	Fatty liver, sev.
11	1.35	39.0	433	3, 4	1, 1	C. D. obstr., bil. cirrhosis
82	0.21	52.5	308	0, 2	0, 0	Metastatic Ca, sev.
43	0.50	3.5	204	1, 2	2, 2	Serum hepatitis, mod.
61	0.82	55.9	375	2, 3	2, 2	Serum hepatitis, mod.
81	0.47	11.1	446	4, 4	4, 5	Portal cirrhosis, sev.
35	0.77	0.9	559	3, 3	1, 2	C. D. obstr., Ca pancreas
6	0.73	15.6	377	4, 4	1, 1	Portal cirrhosis, sev.
56	0.44	4.7	548	3, 3	0, 0	Infectious hepatitis, mod.
49	--	36.1	515	3, 3	0, 1	Serum hepatitis, mod.
77	0.47	9.0	513	4, 4	1, 1	Serum hepatitis, sev.
74	0.42	22.1	423	4, 4	4, 5	Serum hepatitis, mod.
46	0.50	0.3	230	0, 1	0, 0	Portal cirrhosis, sev.
68	0.35	52.6	638	3, 3	4, 4	Portal cirrhosis, sev.
44	0.35	10.7	510	3, 4	1, 2	Serum hepatitis, mod.
124	0.29	11.0	402	4, 4	4, 4	Portal cirrhosis, sev.
66	0.58	8.7	342	2, 3	0, 1	Serum hepatitis, sev.
64	0.33	0.3	308	3, 4	0, 0	Hodgkin's disease of liver
95	0.82	10.3	240	2, 3	2, 3	Serum hepatitis, mod.
52	0.63	16.6	441	--	--	
5-124	0.19-1.35	0.3-71.0	120-829	--	--	

Gal., I.V. galactose, 60', mg. %.
Hipp., I.V. hippuric acid, Gm.
U.U., Urine urobilinogen, 24 hr., mg.
5 c.c. UCP, 5 c.c. urine coproporphyrin, 24 hr., μg.
CCF, Cephalin-cholesterol flocculation, 24 and 48 hr.
CR, Colloidal red, 24 and 48 hr

are limited by the disproportionate alteration of certain tests in specific diseases, and by the existence of relatively low intercorrelations when jaundice is not a factor. Examples are disproportionate elevation of the thymol turbidity and zinc sulfate in hepatitis, and disproportionate reduction of cholesterol esters and urine urobilinogen in the presence of severe jaundice. The over-all pattern however can be delineated. In general the different function tests most sensitively representing hepatocellular dysfunction per se, such as the galactose and hippuric acid tests, vary similarly. This can be seen from an examination of Table II in which are given the clinical diagnoses as well as results with the battery of liver function tests in thirty-seven cases with one-minute bilirubin values greater than 1.0 mg. per cent. In Table II are listed the original bromsulfalein scores as well as the corrected values obtained by subtracting 27.8 log (one-minute bilirubin) from the former. The cases are arranged in order of increasing magnitude of the corrected bromsulfalein scores. With due allowance for disproportionately abnormal scores on certain tests in specific diseases, one sees that when the corrected bromsulfalein remains elevated the other tests generally follow a similar pattern of abnormality. Cases Z 40, 42, and 44 are examples. Conversely, the greater the adjustment the less abnormal are the other tests of hepatocellular dysfunction. Cases Z 1, 92, and 103 are examples. It will be noted for Z 92 that the bromsulfalein corrected to –5 per cent. A negative value such as this results from applying the average correction we have derived to a bromsulfalein value initially disproportionately low, and should be considered the same as a zero value. The mean values at the bottom of Table II are recorded for descriptive purposes only.

A comparison with the galactose test is of particular value because it is not influenced by the degree of jaundice and is one of the most sensitive function tests. The group of thirty-seven cases was divided into four subgroups on the basis of the corrected bromsulfalein score as follows: A, scores 0 to 7; B, scores 8 to 17; C, scores 18 to 27; and D, scores 28 and over. For each of these subgroups mean values were calculated for the bromsulfalein, corrected bromsulfalein, and galactose tests. The results are recorded in Table III.

TABLE III

SUBGROUP		BSP	CORRECTED BSP	GALACTOSE
A	Number	9	9	9
	Mean	21	4	29
	Range	8-37	0-6	5-66
B	Number	7	7	7
	Mean	25	12	57
	Range	17-34	8-16	29-87
C	Number	11	11	11
	Mean	34	21	50
	Range	21-49	18-26	6-82
D	Number	10	10	10
	Mean	50	31	71
	Range	38-58	28-43	44-124

It is apparent that the corrected bromsulfalein score reflects the degree of abnormality indicated by the galactose scores even though the correspondence is not perfect.

Perhaps one of the best tests of the formula's validity is its accuracy in predicting a normal bromsulfalein value in cases of pure obstructive jaundice, and an abnormal value in cases considered purely obstructive clinically but acutally having appreciable liver involvement. Most patients with common duct obstruction already have some evidence of liver dysfunction by the time they are seen, if sufficiently sensitive tests are used. However, such cases, when not associated with advanced malignancies or prolonged obstruction, generally have approximately normal corrected bromsulfalein scores. Cases Z 1 and Z 90 are examples. The following two brief summaries illustrate particularly well the accuracy of the corrected bromsulfalein score in predicting absence or presence of primary liver involvement.

PATIENT I. O.—aged 61; illness, six weeks; jaundice, four weeks. Surgery: Whipple pancreatectomy. Diagnosis: small Ca common bile duct.

TEST RESULTS

T.T.	5.0 units	Alk. p-tase	60 KA units
Z.T.	7.2 units	Cholesterol	269 mg. %
CCF	1+, 2+	% CE	10
U.U.	3.2 mg./day	Fecal U. (random spec.)	8 E.U.

Tot. Bi.	31.8 mg. %
1 min. Bi.	17.9 mg. %
BSP	38%
Corrected BSP	3%

PATIENT E. H.—aged 72; Hodgkin's disease. Diagnosis after tests: common duct obstruction by lymph nodes. Autopsy one week later: extensive infiltration of liver.

TEST RESULTS

T.T.	3.5 units	Alk. p-tase	57 KA units
Z.T.	3.3 units	Cholesterol	234 mg. %
CCF	0, 0	% CE	28
U.U.	0.2 mg./day	Hippuric acid	0.33 Gm.
A/G	4.3/2.2 Gm. %	Galactose	64 mg. %

Tot. Bi.	13.6 mg. %
1 min. Bi.	8.4 mg. %
BSP	58%
Corrected BSP	32%

A third indication of the validity of the correction is the fact that the distribution of corrected bromsulfalein values for the thirty-seven cases falling above the dividing line in Fig. 2 follows closely the distribution of obtained bromsulfalein values for the fifty-seven cases falling below the line. In Fig. 5 these distributions are plotted and contrasted with the distribution of uncorrected bromsulfalein values. It is evident that the formula over-corrects slightly. For comparison we have also plotted distributions of the galactose and hippuric

acid values obtained on the same patients. Surprisingly these curves indicate significant association between the bilirubin and hippuric acid values. The actual correlation coefficient in the region above a one-minute bilirubin of 1.0 mg. per cent was –0.43, a value significantly different from zero but not from 0.59 (ignoring sign). Below 1.0 mg. per cent the correlation was –0.29 (barely significant). Uncorrected this test is therefore no more valid in the presence

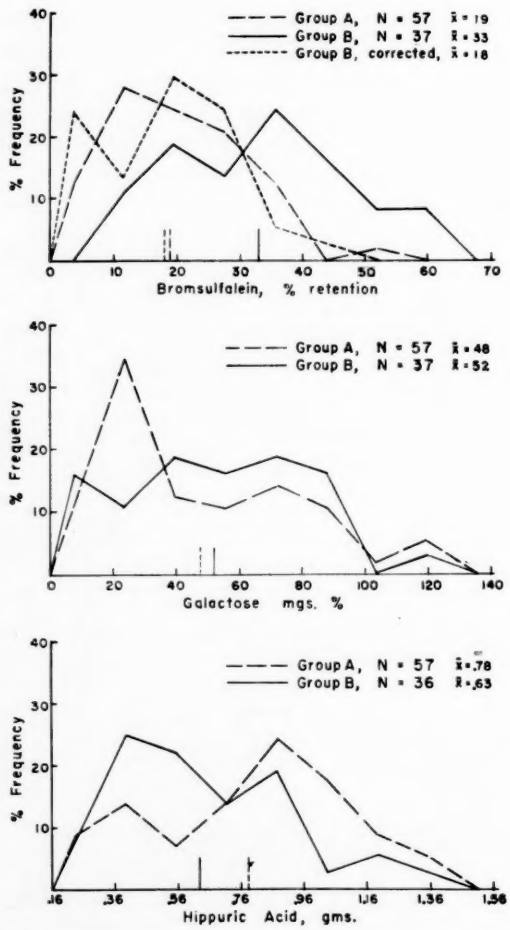
Group A, N = 57 x̄ = 19
Group B, N = 37 x̄ = 33
Group B, corrected, x̄ = 18
% Frequency
Bromsulfalein, % retention
Group A, N = 57 x̄ = 48
Group B, N = 37 x̄ = 52
% Frequency
Galactose mgs. %
Group A, N = 57 x̄ = .78
Group B, N = 36 x̄ = .63
% Frequency
Hippuric Acid, gms.

Fig. 5.—Frequency distributions of bromsulfalein, galactose, and hippuric acid test results.

Group A: Patients with one-minute bilirubin ≤ 1.0 mg. per cent.

Group B: Patients with one-minute bilirubin > 1.0 mg. per cent.

The mean values, $\bar{x}$ are indicated by vertical lines extending up from the abscissa.

of marked jaundice than the uncorrected bromsulfalein. These findings will be presented in detail in a later report. The corresponding correlation coefficients between galactose and one-minute bilirubin were 0.20 and 0.09 respectively, both insignificant.

Precautions in the Use of the Formula.—The formula is an empirical one, applicable only in the range considered in its derivation. However, being empirical, it does not depend for its validity upon the correctness of any theory as to the mechanism of bromsulfalein elevation in the presence of jaundice. It gives an average correction only and should be interpreted as an approximate figure. One can hypothecate unusual clinical circumstances where it may break down. Since it is based on an unselected group of hospital patients, it should be applicable to most cases of jaundice. In the present sample there were no cases of severe jaundice of very short duration. The formula should therefore not be applied to such cases.

SUMMARY

The relationship of bromsulfalein retention and blood bilirubin concentration has been studied in detail.

The 1.0 mg. per cent level of the one-minute bilirubin divides the scattergraph into two regions. Below this point there is no significant association of bromsulfalein and one-minute bilirubin. Above this point the two variables are significantly correlated, the coefficient of correlation between the bromsulfalein and log one-minute bilirubin being 0.59.

The bromsulfalein and total bilirubin are similarly related, 2.0 mg. per cent being the level dividing the graph into two corresponding regions.

Below the one-minute bilirubin level of 1.0 mg. per cent or total bilirubin level of 2.0 mg. per cent the bromsulfalein test may be interpreted directly as in nonjaundiced patients.

From the regression equation relating the bromsulfalein to the one-minute bilirubin in the region of significant association, an average correction was derived which allows one to calculate the bromsulfalein value that would have been obtained had significant jaundice not been present.

A table and a nomograph which facilitate this adjustment are included.

REFERENCES

1. Zieve, L., Hill, E., and Nesbitt, S.: Studies of Liver Function Tests. I. A Combined Intravenous Bromsulfalein-Hippuric Acid-Galactose Test, J. LAB. & CLIN. MED. **36**: 705, 1950.
2. Watson, C. J.: Some Newer Concepts of the Natural Derivatives of Hemoglobin, Blood **1**: 99, 1946.
3. Schwartz, S., Zieve, L., and Watson, C. J.: A Simplified Method for the Determination of Urinary Coproporphyrin and an Evaluation of Factors Involved in the Analysis. To be published.
4. Ducci, H.: Colloidal Red Test for Study of Hepatic Dysfunction, J. LAB. & CLIN. MED. **32**: 1273, 1947.

THE EFFECT OF AUREOMYCIN ON UROBILINOGEN FORMATION AND THE FECAL FLORA

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IT HAS been demonstrated that aureomycin has a growth-suppressing effect upon certain of the intestinal flora[1, 2, 3] and a preliminary report described the markedly reducing effect of aureomycin on urobilinogen formation.[4] A slight or moderate effect of this type had been noted previously following sulfaguanidine,[5] and, more recently, Kolb and Oppenheim[6] have also observed a marked inhibition of urobilinogen formation in two patients, one receiving aureomycin, the other Chloromycetin. Since the conversion of bilirubin to urobilinogen is believed to be mediated by the intestinal flora, it was thought that a study of urobilinogen in bile and feces in a group of patients receiving aureomycin might provide fundamental information concerning urobilinogen formation. In particular it was considered that such a study might be instructive with respect to validity of the concept advanced in recent years by Baumgärtel[7-11] that mesobilirubinogen is formed by reduction of bilirubin in the liver, stercobilinogen by the bacterial reduction of bilirubin in the colon. This concept in other words would revive the hepatogenous theory of urobilinogen formation and give it equal weight with the enterogenous theory, the latter having been most widely acepted. The pertinent literature has been considered recently[12] and need not be repeated at present.

EXPERIMENTAL

Feces Studies.—A pilot experiment was first undertaken in seven patients receiving aureomycin therapy. In these patients fecal urobilinogen measurements[13] were made during and either before or after aureomycin therapy. The results are seen in Table I. It is noted that although the feces urobilinogen values were within normal range both before and after the aureomycin therapy, a marked reduction of the urobilinogen, sometimes to nil, occurred while the patient was receiving aureomycin. The amounts of fecal urobilinogen were comparable in most instances to those seen in patients with complete extrahepatic biliary obstruction.

A somewhat more detailed study was then carried out in three additional patients. In these the fecal urobilinogen content was determined and the feces bilirubin was determined in semiquantitative fashion by the barium strip method.[14] In addition, bacteriologic studies were carried out on the fecal samples. It is seen in Table II that the feces of Case J. prior to aureomycin therapy contained 160 mg. of urobilinogen, and no demonstrable bilirubin. Twenty hours after therapy was begun only 3 mg. of urobilinogen and 1+ bilirubin were present. At this time typical *Escherichia coli* and clostridia were still

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TABLE I. EFFECT OF AUREOMYCIN ON FECAL UROBILINOGEN—PILOT STUDIES

PATIENT	BEFORE AUREOMYCIN (MG./DAY)	DURING AUREOMYCIN (MG./DAY)	AFTER AUREOMYCIN (MG./DAY)
1. E. S.	28.4	0	
	52.4	0.224	52.4
2. J. D.	128.1	0.103	
3. H. M.	108.7	0.184	357.0
4. S. C.	134.6	0.096	
5. J. M.		2.24	
		0.344	
6. R. H.		11.6	
7. M. B.		17.14	

TABLE II. EFFECT OF AUREOMYCIN ON UROBILINOGEN FORMATION AND FECAL FLORA—CASE J.

DATE	FECES UROBILINOGEN (MG./100 GM.)	BILIRUBIN	FLORA	DURATION OF AUREOMYCIN THERAPY
5/20	160.0	0		———
5/21	3.0	1+	Typical E. coli and clostridia	20 hr.
5/27	0.5	2+	Aerogenes; no coliforms	6 days
6/11	61.0	0	Probable E. coli (all coliform)	Discontinued

demonstrable. Six days after therapy was commenced, the feces contained but 0.5 mg. urobilinogen, and bilirubin was readily demonstrable. No coliform organisms could be identified at this time. Later, when coliform organisms were again abundant, bilirubin was not demonstrable and urobilinogen was plentiful. In Case R. K. (Table III), the same phenomenon was observed except that here very little bilirubin was noted.

TABLE III. EFFECT OF AUREOMYCIN ON UROBILINOGEN FORMATION AND FECAL FLORA—CASE R. K.

DATE	FECES UROBILINOGEN (MG./100 GM.)	BILIRUBIN	FLORA	DURATION OF AUREOMYCIN THERAPY
5/10	96.0	0	Normal	———
5/12	9.0	Tr.	No coliforms or colon-aerogenes	2 days
5/13	1.3	0		3 days

The same general pattern of reduced urobilinogen formation following aureomycin therapy has now been observed in a total of fifteen cases. Within five days, usually sooner, after onset of therapy, the fecal urobilinogen markedly diminishes or disappears almost completely, to return rather rapidly after discontinuance of treatment.

In Case C. A. (Table IV), the simple quantitative Ehrlich method[15] was used in addition to the petroleum ether extraction technique for urobilinogen.[8] This patient had a refractory endocarditis and had been on aureomycin therapy for forty-five days (2 Gm. daily) at the outset of the urobilinogen observations. Bacteriologic studies up to sixty-eight days after the beginning of therapy revealed that there were no typical *E. coli* in the fecal flora, but that colon aerogenes intermediates and nonproteolytic clostridia were present.

A rather marked discrepancy may be noted in Table IV between the Ehrlich units per 100 grams of feces and the milligrams of urobilinogen per 100 grams.

TABLE IV. EFFECT OF AUREOMYCIN ON UROBILINOGEN FORMATION AND FECAL FLORA—CASE C. A. (SUBACUTE BACTERIAL ENDOCARDITIS)

DATE	FECES: EHRLICH UNITS PER 100 GM.	FECES: UROBILINOGEN (MG./100 GM.)	FECES: BILIRUBIN	FLORA	DURATION OF AUREOMYCIN THERAPY
2/29				Aerobacter; no E. coli or coliform	48 days
2/7	80	10.0	Trace	Nonproteolytic clostridia; colon	55 days
3/10	90	12.0	2+	Aerogenes intermediates; no typical E. coli	58 days
3/20	96*	12.0			68 days
3/26					Discontinued
4/10	80	50.0	0		
4/16	57	29.0	0		

*Crystalline stercobilin or urobilin could not be isolated; a dextrorotatory urobilin was observed.

While the value for Ehrlich units is regularly from 10 to 40 per cent larger than that for milligrams of urobilinogen by the extraction procedure,[15, 16] the present discrepancy was considerably greater. Because of this, an attempt was made to isolate the urobilin present by means of the method previously described for stercobilin.[17] It was evident, however, that the pigment in the final solution was more labile and difficult to crystallize than stercobilin, and in fact, crystalline material could not be obtained. Nevertheless the concentration in the final chloroform solution was adequate for polarimetry. The absorption spectrum was compared* with that of stercobilin and urobilin IX,a, and was found identical with the latter, in accord with previous observations.[12] The two solutions were then adjusted to the same reading with the Evelyn colorimeter and their optical rotation was measured. While the solution of crystalline stercobilin exhibited characteristic levorotation, with a polarimeter reading of (–) 0.45, that of the urobilin from Case C. A. was dextrorotatory, with a reading of (+) 0.2; zero point for the solvent, $CHCl_3$ was (+) 0.2. This was subtracted to give the foregoing values. No attempt is made to calculate specific rotation in the lack of a crystalline material.

The same large discrepancy between the simple Ehrlich method and the fecal urobilinogen in milligrams is also noted in the serial data from two additional cases, E. R. and F. K., as shown in Figs. 1 and 2 respectively. This is particularly striking shortly after cessation of aureomycin therapy in Case F. K. (Fig. 2), and in fact, on the second day thereafter, there were but 7 mg. of urobilinogen per 100 Gm., by the quantitative method,[13] as contrasted with 272 Ehrlich units per 100 Gm. by the simple Ehrlich procedure.[15] Nevertheless it was evident that the latter was due in the main, at least, to a urobilinogen. The sample was subjected to the method of isolating crystalline stercobilin, and in this instance 8 mg. of crystalline material were obtained. After recrystallization of the hydrochloride from $CHCl_3$ the melting point was 126 to 128° C., quite similar

*We are indebted to Dr. S. Schwartz for the spectroscopy and polarimetry.

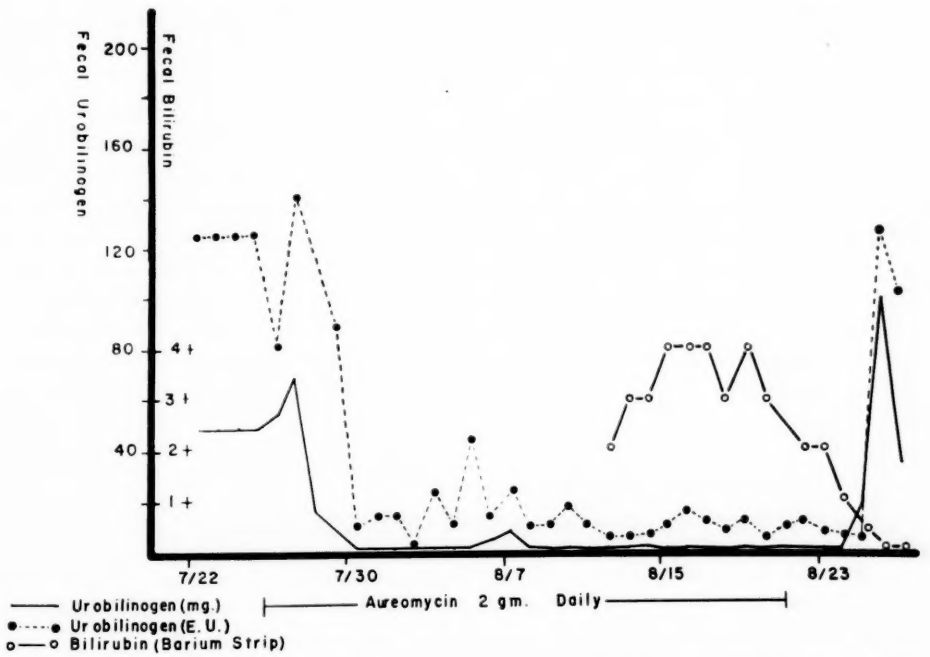

Fig. 1.—Fecal urobilinogen in milligrams per 100 Gm. and Ehrlich units (E.U.) per 100 Gm., and bilirubin, in Case E. R.

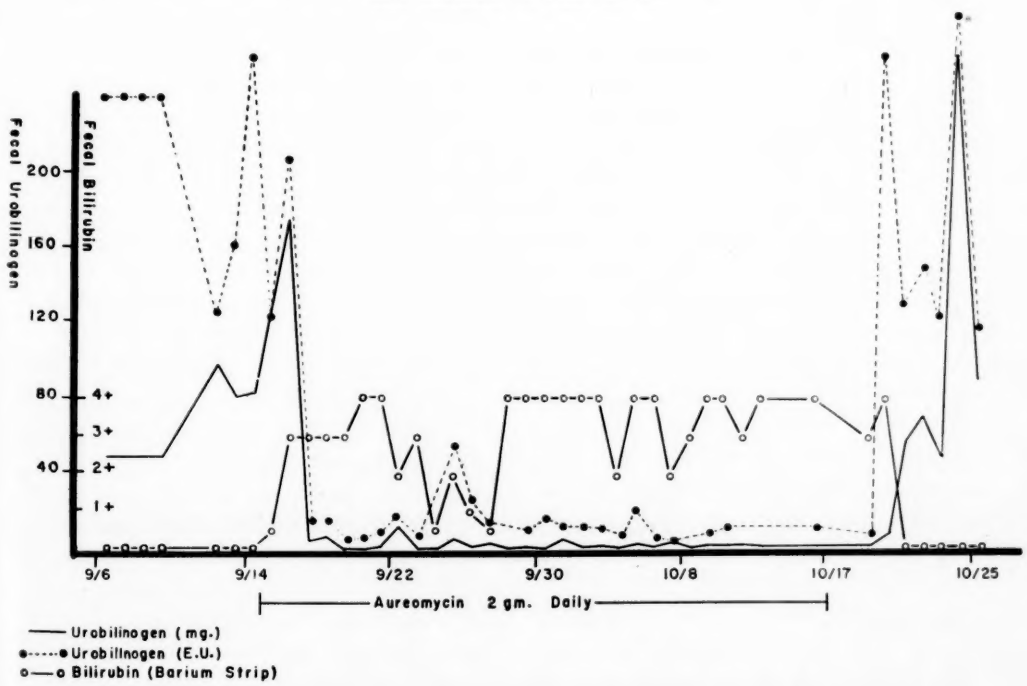

Fig. 2.—Fecal urobilinogen in milligrams per 100 Gm. and Ehrlich units (E.U.) per 100 Gm., and bilirubin, in Case F. K.

to that of crystalline stercobilin hydrochloride.[18] This compound, however, was dextrorotatory: $[a]^{20°}_{589\ m\mu} = +4000$ (in $CHCl_3$); in other words about the same as stercobilin is levorotatory. This substance appears to be similar to if not identical with the D-urobilin previously isolated from infected human fistula bile.[19]

Since it appeared that a principal difference in explaining the discrepancy between the two methods might be a lesser solubility of the D-urobilinogen in petroleum ether, the crystalline material was dissolved in dilute sodium hydroxide, ferrous sulfate was added, and the reduction by ferrous hydroxide was carried out in the usual way.[20] After one hour the filtrate was subjected to the simple Ehrlich procedure[15] as well as the quantitative, petroleum ether extraction method.[13] The results, however, were identical, as a consequence of which it appears that the D-urobilinogen as it occurs in the original ferrous hydroxide filtrate from the feces is not as soluble in petroleum ether as it is after it has been prepared from crystalline material.

Bile Studies.—The total urobilinogen content of bile samples was measured in ten patients while they were receiving aureomycin. In four of these the urobilinogen value was also determined either before or after aureomycin. The bile was obtained according to the Lyon method[21] by duodenal drainage following instillation of magnesium sulfate solution. As noted in Table V the bile samples in seven of the ten individuals studied contained no urobilinogen during the period of therapy and in the other three the amounts were negligi-

TABLE V. EFFECT OF AUREOMYCIN UPON BILIARY UROBILINOGEN

PATIENT	TYPE OF BILE*	BEFORE AUREOMYCIN (MG. U/100 C.C.)	TYPE OF BILE	DURING AUREOMYCIN (MG. U/100 C.C.)	TYPE OF BILE	AFTER AUREOMYCIN (MG. U/100 C.C.)
1. B. C.			A + B	0		
			C	0		
2. J. G.			C	0		
3. S. A.			A	0		
			C	0		
4. B. C.			A	0		
			C	0		
5. G. H.			A + B	0.012		
			C	0		
6. C. M.			A + B	0.014	A + B	1.63
			C	0.005	C	0.13
7. M. J.	A + B	2.76	A + B	0	A + B	15.4
	C	0.28	C	0	C	0.70
8. H.K.	A + B	1.24	A + B	0.019	A + B	3.42
	C	0.56	C	0.014	C	1.18
9. W. T.			A + B	0		
			C	0		
10. M.W.	A + B	2.97	A + B	0		
	C	0.81	C	0		

*A, B, or C, or combinations, as obtained by Lyon duodenal drainage.[21]

ble. Where measurements were made either before or after the aureomycin, a uniform reduction of urobilinogen content was noted while it was being given. Of a total of eighty-five normal individuals and patients in whom urobilinogen measurements of both "A + B" and "C" biles were made, the only instances in which no urobilinogen whatever was found were in patients receiving aureomycin, as listed in Table V. The values for urobilinogen in the control series ranged from 0.13 to 15.4 milligrams per 100 c.c. bile. Thus, it is evident that with a suppression of urobilinogen formation in the intestine, there is a concomitant disappearance of urobilinogen from the bile.

DISCUSSION

As a result of the earlier classical experiments of Friedrich v. Müller,[22] and the later in vitro conversions of bilirubin to urobilinogen by fecal bacteria carried out by Kämmerer and Miller,[23] the enterogenous theory of urobilinogen formation has become widely accepted. The various theories which have been advanced, together with additional evidence supporting the enterogenous one, have been considered recently in some detail.[12] The results of the present study are especially significant with respect to Baumgärtel's papers which have appeared within the past few years and, as mentioned at the outset, postulate that the reduction of bilirubin to mesobilirubinogen is hepatic while that of bilirubin to stercobilinogen is bacterial, occurring in the colon. These papers overlooked previously described evidence as to the transition of mesobilirubinogen to stercobilinogen in the human intestinal tract, or in feces incubated outside the body.[24] The effect of aureomycin in largely eliminating coliform organisms from the feces and urobilinogen completely or almost completely from the feces and bile is in agreement with the enterogenous concept and does not support Baumgärtel's belief that mesobilirubinogen is formed in the liver and excreted in the bile. One might consider the possibility that aureomycin rather selectively depresses liver function in such a fashion that a reduction of bilirubin to mesobilirubinogen no longer occurs, but there is nothing to suggest such a possibility. Studies recently carried out by one of us (V.M.S.), to be described separately, reveal that terramycin is even more efficient in eliminating the fecal flora necessary to the reduction.

Kämmerer and Miller[23] ascribed the reduction of bilirubin in the colon to a symbiotic activity of *E. coli* and a terminal spored or "tennis racket" type of anaerobe. Baumgärtel[9] believes that a similar anaerobe, which he identifies as *"Bacillus putrificus verrucosus,* a normal inhabitant of the human cecum," reduces cystine to cysteine. According to Baumgärtel hydrogen is then transferred from cysteine to bilirubin by the dehydrogenase of *E. coli,* and the reduction to stercobilinogen is thus effected. The present bacteriologic data tend to support the belief that coliform organisms are of prime importance in the reduction. As a rule they disappeared quite regularly as the urobilinogen values approached zero; nevertheless it is interesting to note in Table II that *E. coli* and clostridia were both still present when the urobilinogen had declined to 3.0 mg. per 100 grams. This, however, was but twenty hours after beginning

aureomycin. Several days later the value was 0.5 mg. per 100 mg., and at this time coliform organisms and clostridia were not demonstrable. The data in Table IV suggest that atypical coliform organisms or intermediates may be able to reduce bilirubin to a dextrorotatory urobilin, but not to stercobilin. The significance of this finding is not clear, but it may be recalled that the incubation of mesobilirubinogen with infected human fistula bile was followed by the formation of a dextrorotatory urobilin.[25] The reappearance in the feces of atypical or intermediate coliform organisms, shortly after discontinuance of aureomycin, may explain the occurrence of relatively large amounts of dextrorotatory urobilin as in Case F. K. (Fig. 2). Further studies are needed of the discrepancy between the values obtained with the simple Ehrlich procedure and the petroleum ether extraction method, with special reference to the significance of the dextrorotatory compound.

The possibility exists and deserves exploration, that at least some of the discrepancy between the two methods, as previously discussed,[15, 16] is on the basis of a dextrorotatory urobilin, not readily extracted by petroleum ether.

From a practical standpoint it is clear that no reliance whatever can be placed on fecal or urinary urobilinogen studies in cases of jaundice or liver disease, when aureomycin is being used or has been used recently. Although data have not been included in the present report, many instances now have been observed in which urobilinogen has rapidly and completely disappeared from the urine, following initiation of aureomycin therapy.

SUMMARY AND CONCLUSIONS

Aureomycin in therapeutic doses causes a rapid decline, to negligible amounts, of the urobilinogen in the feces, bile, and urine. Bilirubin becomes easily demonstrable in the feces. This is associated with a disappearance of coliform organisms and diminution or disappearance of clostridia, in the feces. No reliance can be placed on urobilinogen studies when aureomycin is being given.

The disappearance of urobilinogen from the feces and bile following aureomycin lends strong support to the enterogenous and bacterial concept of urobilinogen formation, the term urobilinogen being employed to include all of the known types, i.e., mesobilirubinogen, stercobilinogen, and D-urobilinogen. This finding does not support Baumgärtel's belief that mesobilirubinogen is formed in the liver.

Under certain circumstances, such as long-continued aureomycin therapy, or recent discontinuance of aureomycin, a much greater than usual discrepancy has been observed between the values obtained with the simple Ehrlich aldehyde procedure, and the quantitative method for urobilinogen depending on petroleum ether extraction. This appears to depend on the presence of a dextrorotatory urobilinogen which in the crude state is much less readily extracted by petroleum ether. The possibility is considered that its formation is due to abnormal or atypical coliform organisms, or intermediate strains occurring as a result of the aureomycin therapy.

REFERENCES

1. Rose, H. M., and Kneeland, Y.: Aureomycin in the Treatment of Infectious Disease, Am. J. Med. **7**: 532, 1949.
2. Harvey, J. C. Mirick, G. S., and Schuab, I. G.: Clinical Experience With Aureomycin, J. Clin. Investigation **28**: 987, 1949.
3. Dearing, W. H., and Heilman, F. R.: The Effect of Aureomycin on the Bacterial Flora of the Intestinal Tract of Man: a Contribution to Preoperative Preparation, Proc. Staff Meet., Mayo Clin. **25**: 87, 1950.
4. Sborov, V. M., Jay, A. R., and Watson, C. J.: The Effect of Aureomycin on Urobilinogen Formation and the Fecal Flora, Proc. Central Soc. Clin. Research **22**: 70, 1949.
5. Greenblatt, I. J., and Greenblatt, A. P.: The Effects of Sulfaguanidine on Urobilinogen and Urobilin Metabolism, Arch. Biochem. **7**: 87, 1945.
6. Kolb, F. O., and Oppenheim, D. J.: Disappearance of Urobilinogen During Antibiotic Therapy, Bull. New Eng. M. Center **12**: 31, 1950.
7. Baumgärtel, T.: Untersuchungen über die Rolle der enteralen Gallenfarbstoffreduktion im enterohepatischen Cystinkreislauf, Klin. Wchnschr. **22**: 92, 1943.
8. Baumgärtel, T.: Zur Kenntnis der biologischen Erythrocytolyse, Klin. Wchnschr. **22**: 457, 1943.
9. Baumgärtel, T.: Zur Kenntnis der biologischen Gallenfarbstoffreduktion, Ztschr. f. d. ges. exper. Med. **112**: 459, 1943.
10. Baumgärtel, T.: Wo entsteht das Urobilinogen? Münch. med. Wchnschr. 407, 1944.
11. Baumgärtel, T.: Zur Acholie der Faeces, Klin. Wchnschr. **24-25**: 315, 1947.
12. Watson, C. J.: Urobilin and Stercobilin, The Harvey Lectures, series XLIV, 1948-49, 41.
13. Watson, C. J.: Studies of Urobilinogen: Improved Method for Quantitative Estimation of Urobilinogen in Urine and Feces, Am. J. Clin. Path. **6**: 458, 1936.
14. Watson, C. J., and Hawkinson, V.: Semiquantitative Estimation of Bilirubin in Urine by Means of Barium-Strip Modification of Harrison's Test, J. LAB. & CLIN. MED. **31**: 914, 1946.
15. Watson, C. J., Schwartz, S., Sborov, V., and Bertie, E.: Studies of Urobilinogen. V. A Simple Method for the Quantitative Recording of the Ehrlich Reaction as Carried Out With Urine and Feces, Am. J. Clin. Path. **14**: 605, 1944.
16. Watson, C. J., and Hawkinson, V.: Studies of Urobilinogen. VI. Further Experience With the Simple Quantitative Ehrlich Reaction. Corrected Calibration of the Evelyn Colorimeter With a Pontacyl Dye Mixture in Terms of Urobilinogen, Am. J. Clin. Path. **17**: 108, 1947.
17. Watson, C. J.: An Improved Method for the Isolation of Crystalline Stercobilin, J. Biol. Chem. **105**: 469, 1934.
18. Watson, C. J., and Schwartz, S.: I. Nature of Urobilin Obtained After Amalgam Reduction of Human Fistula Bile, Proc. Soc. Exper. Biol. & Med. **49**: 636, 1942.
19. Watson, C. J., and Schwartz, S.: II. Isolation of a Dextrorotatory Urobilin From Human Fistula Bile, Proc. Soc. Exper. Biol. & Med. **49**: 641, 1942.
20. Watson, C. J., Schwartz, S., and Sborov, V.: Studies of Urobilinogen. IV. The Quantitative Determination of Urobilinogen by Means of the Evelyn Photoelectric Colorimeter, Am. J. Clin. Path. **14**: 598, 1944.
21. Lyon, B. B. V.: Nonsurgical Drainage of the Gall Tract, Philadelphia, 1923, Lea and Febiger.
22. v. Müller, F.: Ueber Ikterus, Jahresb. d. schles. Gesellsch. f. vaterl. Kult., Bresl. Med. Sekt., 1892, p. 1.
23. Kämmerer, H., and Miller, K.: Zur enterogenen Urobilinbildung, Deutsches Arch. f. klin. Med. **141**: 318, 1923.
24. Watson, C. J., Sborov, V., and Schwartz, S.: IV. Formation of (Laevorotatory) Stercobilin From Mesobilirubinogen in Human Feces, Proc. Soc. Exper. Biol. & Med. **49**: 647, 1942.
25. Watson, C. J., Schwartz, S., and Sborov, V.: III. Formation of d-urobilin From Mesobilirubinogen in Human Bile, Proc. Soc. Exper. Biol. & Med. **49**: 643, 1942.

THE IN VITRO SENSITIVITY TO EIGHT ANTIBIOTICS OF STREPTOCOCCI AND STAPHYLOCOCCI RECOVERED FROM CASES OF BACTERIAL ENDOCARDITIS

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VERY little is known of the effectiveness of the newer antibiotics in the treatment of bacterial endocarditis. One of the factors which will necessarily play a role in assessing the value of these drugs in actual therapy is the in vitro sensitivity of the bacteria which commonly cause this disease. For that reason we have determined the minimal inhibiting concentration of eight antibiotics for eleven strains of alpha streptococci *(Streptococcus viridans)*, two strains of Group D streptococci, and three strains of staphylococci recovered from cases of subacute bacterial endocarditis. The antibiotics are: penicillin, streptomycin, aureomycin, chloramphenicol, terramycin, neomycin, bacitracin, and polymyxin. It is the purpose of this paper to report the results.

METHOD

A twofold serial dilution test in broth was employed, using twelve 100 by 13 mm. tubes in each test. The twelfth tube was a control containing no antibiotic. Each tube contained 0.5 c.c. of dilutio nof antibiotic solution and 1.5 c.c. of an appropriate dilution of the culture of the organism being tested. In most instances a dilution of 1:50 in broth was used. Yeast-beef broth (Penassay broth, Difco) was employed for penicillin, aureomycin, chloramphenicol, terramycin, bacitracin, neomycin, and polymyxin. Tryptone broth (Difco) 1 per cent was used for streptomycin. In most instances 5 per cent defibrinated rabbit blood was added to each tube before incubation.

The antibiotics were prepared from crystalline material supplied by the manufacturer.* In the case of penicillin, streptomycin, aureomycin, chloramphenicol, neomycin, and polymyxin, standard laboratory strains were tested as a control against the potency of the antibiotic solution. The range of dilutions employed varied according to the antibiotic and the approximate estimated sensitivity of the unknown organism as judged by previous experience. The tests were incubated at 37° C. for twenty-four hours except for aureomycin, which was incubated for sixteen hours. The last tube in which no macroscopic growth occurred was designated the minimal inhibitory concentration.

RESULTS

The tests are summarized in Table I. It is readily seen that the strains of *Streptococcus viridans* not belonging to Group D were more susceptible to the action of penicillin than to any of the other antibiotics. The minimal inhibiting concentration of penicillin ranged from 0.006 to 0.06 μg per cubic centimeter. Bacitracin, aureomycin, and terramycin were all very effective, though

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*Penicillin and streptomycin were supplied by Merck & Co., Inc., aureomycin by Lederle Laboratories, chloramphenicol by Parke, Davis & Co., terramycin by Chas. Pfizer & Co., neomycin by The Upjohn Company, polymyxin by Burroughs Wellcome & Co., and bacitracin through the courtesy of Dr. William A. Altemeier.

TABLE I. IN VITRO ANTIBIOTIC SENSITIVITY OF BACTERIA RECOVERED FROM CASES OF BACTERIAL ENDOCARDITIS

PATIENT	ORGANISMS	MINIMAL INHIBITING CONCENTRATION (μG/C.C.)							
		PNC	AMC	CMP	TMC	NMC	SMC	BCN	PMX
W. P.	Alpha str.	0.03	0.2	6.25	0.5	12.5	1.2	0.012	>100
M. W.	Alpha str.	0.06	0.1	3.12	0.5	0.012	0.6	0.4	>100
H. N.	Alpha str.	0.007	0.025	0.78	0.06	3.12	>1250	0.012	>100
H. O.	Alpha str.	0.007	0.39	1.56	0.5	<0.1	1.25	0.05	100
R. Wh.	Alpha str.	0.06	0.2	1.56	0.25	3.12	1.25	<0.012	25
A. W.	Alpha str.	0.015	0.1	3.12	0.12	0.1	0.01	0.05	>100
E. B.	Alpha str.	<0.007	<0.012	6.25	0.125	>12.5	<1.25	0.8	100
C. R.	Alpha str.	<0.015	<0.012	3.12	0.5	>12.5	2.5	0.1	100
R. W.	Alpha str.	0.007	0.05	1.56	0.25	>25	5.0	0.05	100
H. L.	Alpha str.	0.015	0.2	3.12	1.0	1.56	1.25	0.78	12.5
M. C.	Alpha str.	<0.007	0.1	3.12	0.5	12.5	2.5	3.12	>100
C. Y.	Group D str.	7.5	25	>12.5	>31.2	>25	2.5	1.56	>100
D. W.	Group D str.	7.5	>12.5	>12.5	>31.2	>25	2.5	1.56	>100
J. C.	S. au. hem. Coag. neg.	0.015	0.1	6.25	0.5	0.39	0.078	0.5	3.12
S. B.	S. au. hem. Coag. pos.	0.06	0.2	>12.5	0.5	3.12	0.31	0.78	50
T. Q.	S. au. hem. Coag. pos.	7.5	0.012	>12.5	1.0	12.5	0.08	0.78	50

Alpha str., Alpha streptococcus.
S. au. hem., *Staphylococcus aureus* hemolyticus.
Coag., Coagulase.
PNC, Crystalline penicillin G; AMC, aureomycin; CMP, chloramphenicol; TMC, terramycin; NMC, neomycin; SMC, streptomycin; BCN, bacitracin; PMX, polymyxin B.

less so than penicillin. Chloramphenicol was moderately effective. There was a wide strain difference in sensitivity to neomycin, the inhibiting concentration varying from 0.01 to more than 25 micrograms. All the strains were highly resistant to polymyxin. Most of the strains proved sensitive to streptomycin. One culture requiring 1250 μg per cubic centimeter for growth inhibition was recovered from a patient given the drug for a month with the mistaken diagnosis of tuberculosis.

Two Group D streptococci were available for study. In addition to the well-known resistance of this organism to penicillin, these two strains proved resistant to aureomycin, chloramphenicol, terramycin, neomycin, and polymyxin. They were fairly susceptible to streptomycin and bacitracin.

The three specimens of *Staphylococcus aureus* proved susceptible to 1 μg or less of bacitracin, terramycin, streptomycin, and aureomycin. One of the strains was highly sensitive to penicillin, one moderately sensitive, and one extremely resistant. None of the cultures was sensitive to neomycin, chloramphenicol, or polymyxin.

DISCUSSION

It is generally agreed[1-4] that the in vitro sensitivity to penicillin of the infecting streptococcus or staphylococcus plays an important role in determining proper therapy of bacterial endocarditis with this antibiotic. When the organism is susceptible to the action of penicillin, one of a number of therapeutic dosage schedules will usually lead to eradication of the infection. When the organism is resistant in vitro, penicillin in conventional dosage will not suffice clinically.

There is very little reported experience in the treatment of bacterial endocarditis with other antibiotics. Streptomycin has been employed effectively in a few instances.[5, 6, 7] Aureomycin and the newer antibiotics have been used hardly at all.

In order for bacteria to be eliminated from a valvular vegetation by a chemotherapeutic agent, the following conditions must obtain: (a) the organism must be susceptible to the action of the agent; (b) enough of the drug must penetrate into the vegetation to provide a bactericidal concentration; (c) the toxicity of the drug must be sufficiently low that enough can be given to accomplish (b). Since no method exists for determining the ratio of plasma concentration of an antibiotic to its concentration in a vegetation, we have no way of knowing what actually constitutes an adequate blood level in treating any given patient. It is not surprising, therefore, that so many different penicillin dosage schedules have been advocated in this disease.

It will take years before the place of the newer antibiotics in the treatment of endocarditis can be established. From the data presented in this paper it would appear that streptomycin, aureomycin, terramycin, and perhaps some of the others may be effective in certain instances in which penicillin is not adequate.

SUMMARY

The in vitro sensitivity to penicillin, streptomycin, aureomycin, chloramphenicol, terramycin, neomycin, bacitracin, and polymyxin of sixteen strains of streptococci or staphylococci from cases of bacterial endocarditis was measured.

Eleven strains of *Str. viridans* not belonging to Group D were highly susceptible to the action of penicillin. Aureomycin, terramycin, bacitracin, and streptomycin were slightly less effective. There was considerable strain variation in susceptibility to streptomycin and neomycin. Chloramphenicol was less effective, and polymyxin entirely ineffectual.

Two strains of Group D streptococci proved resistant to penicillin, aureomycin, terramycin, chloramphenicol, neomycin, and polymyxin. They were fairly sensitive to bacitracin and streptomycin.

Three strains of *Staph. aureus* were susceptible to streptomycin, aureomycin, terramycin, and bacitracin. They were resistant to chloramphenicol and polymyxin. There were strain differences in reaction to penicillin and neomycin.

REFERENCES

1. Christie, Ronald V.: Penicillin in Subacute Bacterial Endocarditis; Report to the Medical Research Council on 269 Patients Treated in 14 Centres Appointed by the Penicillin Clinical Trials Committee, Brit. M. J. **1**: 1, 1948.
2. Drake, E. H., and Denham, Ralph M.: Therapy of Subacute Bacterial Endocarditis. A Report of 24 Cases Treated With Penicillin, Am. J. M. Sc. **217**: 485, 1949.
3. Friedberg, Charles K.: Revised Criteria for Diagnosis and Treatment of Subacute Bacterial Endocarditis, M. Clin. North America **34**: 769, 1950.
4. Reveley, Hugh: Subacute Bacterial Endocarditis. Laboratory Aspects, Texas State J. Med. **45**: 94, 1949.
5. Priest, Walter S., and McGee, Charles J.: Streptomycin in the Treatment of Subacute Bacterial Endocarditis, J. A. M. A. **132**: 124, 1946.
6. Cressy, Norman L., Lahey, William J., and Kunkel, Paul: Streptomycin in the Treatment of Bacterial Endocarditis. Report of Two Cases, New England J. Med. **239**: 497, 1948.
7. Wallach, Robert, and Pomerantz, Norman: Streptomycin in the Treatment of Subacute Bacterial Endocarditis, New England J. Med. **241**: 690, 1949.

STREPTOMYCIN-RESISTANT COLIFORM BACILLI IN STOOLS OF TUBERCULOUS PATIENTS TREATED WITH INTRAMUSCULAR STREPTOMYCIN

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IN 1947 a study was undertaken to investigate the possible effect of intramuscularly administered streptomycin upon the development of streptomycin-resistant variants among the coliform bacilli of the stool. It was felt that this therapy might influence the ecology of the bowel flora and should be studied because certain of these bacteria produce infection when they leave the colon and invade other parts of the body.

It was soon found that though streptomycin-resistant coliforms were almost uniformly absent from the stools of subjects who had not received streptomycin, they appeared in the feces of an appreciable number of patients given the drug intramuscularly in treatment of tuberculosis. It was of further interest that the resistant variants were found frequently in cases of miliary tuberculosis, tuberculous meningitis, and tuberculous peritonitis, but uncommonly in cases of uncomplicated pulmonary tuberculosis.[1] It is the purpose of this paper to present these and certain related observations.

METHODS AND MATERIAL

Selection of Cases.—The patients studied were hospitalized on the wards of the Cincinnati General Hospital, the Dunham Memorial Hospital (Hamilton County Tuberculosis Sanatorium),* and the Veterans Administration Hospital, Dayton, Ohio.† They included thirty-five cases of nonmiliary pulmonary tuberculosis, twenty-seven cases of miliary tuberculosis, tuberculous meningitis, or both in the same patient, and sixteen cases of tuberculous peritonitis.

The patients with pulmonary tuberculosis all displayed the clinical and roentgenologic picture of the disease; in every instance, the sputum contained bacilli. The cases of miliary tuberculosis were diagnosed on the basis of clinical findings, typical x-ray, and hospital course or autopsy results; in only four cases was the sputum positive for tubercle bacilli. In one case multiple small tubercles were found in a liver biopsy. Tuberculous meningitis was diagnosed on clinical grounds, including examination of the cerebrospinal fluid; in some instances autopsy confirmation was available. Tubercle bacilli were not always found in the spinal fluid. The diagnosis of tuberculous peritonitis was made in most cases on the basis of clinical evidence only, though in a few instances exploratory laparotomy gave confirmatory proof.

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†These patients were studied through the courtesy of Dr. D. E. Nolan, Dr. Morris Thomas and Dr. E. P. Sauer.

Dose of Streptomycin.—Patients in this series received either 1 or 2 Gm. of streptomycin per day. The drug was continued for six weeks in cases of pulmonary tuberculosis and for as long as four months in a few patients. In tuberculous peritonitis, streptomycin was administered for six to twelve weeks. In the cases of miliary tuberculosis and/or tuberculous meningitis, the drug was given for three to six months.

All but three of the patients with pulmonary tuberculosis received 1 Gm. a day. In the miliary-meningitis group, eleven patients were given 1 Gm. a day and twelve 2 Gm. a day, whereas in four the dose varied from 1 to 2 grams. Six of the peritonitis patients received 2 Gm. a day and ten 1 gram.

Stool specimens were obtained, when possible, before, during, and after streptomycin therapy. In a few instances it was not possible to procure specimens prior to treatment. These cases are included in the series because of the virtual absence of streptomycin-resistant coliforms from a large number of stool samples from untreated patients, as described under Results. An effort was made to secure specimens at approximately weekly intervals during therapy, though this effort fell quite wide of the mark. Though in some cases one or more stools were obtained each week, in others several weeks elapsed between samples.

Stool Cultures.—Approximately 0.5 Gm. stool was emulsified in 5 c.c. 0.9 per cent saline solution. One standard 3 mm. loopful of this suspension was then streaked on each of two eosin-methylene blue (EMB) agar plates, one of which was prepared so as to contain 100 μg streptomycin per cubic centimeter of medium (henceforth referred to as the streptomycin plate), and the other no streptomycin (henceforth referred to as the plain plate). The plates were incubated at 37° C., and examined at the end of twenty to twenty-four hours. The approximate number of colonies of coliform bacteria was recorded as + to ++++. Plates showing no growth were reincubated and read at forty-eight and seventy-two hours.

One to twelve colonies were picked from representative plates for determination of their streptomycin resistance by a serial dilution test.

In some cases, twelve colonies selected at random from either the plain or the streptomycin plate were restreaked on another streptomycin plate and incubated overnight at 37°. This technique was employed as a rapid means of determining the approximate number of "sensitive" and "resistant" colonies on a plain plate, since only streptomycin-resistant variants would grow on the streptomycin plate. The selection of 100 μg streptomycin per cubic centimeter was based upon our experience testing individual colonies in the serial dilution test. Practically all the coliforms encountered in stools were inhibited by 5.0 μg streptomycin per cubic centimeter (sensitive strains) or else required at least 156 μg for growth inhibition (resistant strains).

Serial Dilution Tests for Streptomycin Resistance.—0.5 c.c. of solution of streptomycin hydrochloride in sterile distilled water was placed in each of two 13 by 100 mm. tubes. 0.5 c.c. sterile tryptone broth was added to the second tube, and twofold serial dilutions in broth were made until the eleventh tube was reached. 0.5 c.c. sterile broth containing no streptomycin was placed in the twelfth tube. 1.5 c.c. of a 1:100 dilution of an eighteen hour culture of the unknown organism was added to each tube. The tests were incubated at 37° C. for twenty-four hours. The lowest concentration of streptomycin in which no macroscopic growth occurred was recorded as the end point.

Streptomycin solutions of two strengths were employed. The "strong" solution, used in testing strains suspected of being resistant, contained 2,500 μg per cubic centimeter of medium as the final concentration in the first tube of the serial dilution test. The "weak" solution contained 5 μg per cubic centimeter under the same conditions. The solutions were prepared from the sterile vials supplied by the manufacturer* and were not autoclaved or filtered.

As a control upon the streptomycin, a culture of known sensitivity, *Bacillus circulans*, was tested each time unknown strains were measured.

*Kindly donated by Merck & Co., Inc.

Definition of "Sensitive" and "Resistant".—Sensitive strains were those whose growth was inhibited by 5 μg streptomycin or less per cubic centimeter of medium. Highly resistant strains were those requiring from 156 to more than 2,500 μg for growth inhibition. Strains whose growth was inhibited by more than 5 and less than 156 μg were considered moderately resistant.

Identification of Coliforms.—Dark colonies 1 to 3 mm. in diameter exhibiting a metallic sheen on an EMB plate were considered to be *Escherichia coli.* Large mucoid colonies growing on the EMB plates were classified as *Aerobacter aerogenes.* Small white nonmucoid colonies not exhibiting sheen were listed as paracolon bacilli. Further identification of representative colonies was carried out by the IMViC series of tests.[2] These tests are described in standard textbooks of bacteriology, and include the indol, methyl red, Voges-Proskauer, and citrate reactions. *Esch. coli* gives the positive reactions in the first two tests, but negative reactions in the second two. *A. aerogenes* reacts exactly oppositely.

In addition, serologic typing of representative sensitive and resistant *Esch. coli* was carried out.* The "O" groups of Kauffmann[3] were determined.

Determination of Streptomycin in Stool Samples.—Approximately 0.5 Gm. stool was suspended in 10 c.c. normal saline solution and thoroughly agitated. The solution was then cleared by centrifugation. Five cubic centimeters of the supernatant solution were removed with a pipette and placed in a boiling water bath for four minutes, in order to kill any bacteria present. A twofold serial dilution assay for streptomycin was then made, using *B. circulans* as the test organism. The number of micrograms of streptomycin per cubic centimeter of saline solution was then calculated. This figure was multiplied by a factor of 20 to find the number of micrograms per gram of stool.

Because of the many variables which enter into a technique such as this, the results can be considered as only quasi-quantitative.

RESULTS

Absence of Streptomycin-Resistant Coliforms in Stools of Patients Not Receiving Streptomycin.—No resistant coliforms were found among eighty-eight colonies picked from the plain plates of ninety-five specimens from eighty patients who had not received streptomycin. Despite luxuriant growth on the plain plates, no growth was seen on ninety-two of the ninety-three streptomycin plates from the same specimens. In one case, three colonies appeared on the streptomycin plate and proved to require 1,250 μg of the antibiotic for growth inhibition; ten colonies from the plain plate of the same specimen were all inhibited by 2.5 μg or less. In this case, therefore, the resistant forms were present in minute numbers.

Appearance of Streptomycin-Resistant Coliforms in Stools of Patients Receiving Streptomycin Intramuscularly: Importance of the Clinical Type of Tuberculosis.—Table I shows the incidence of the appearance of streptomycin-resistant coliform bacilli in the stools of thirty-five cases of nonmiliary pulmonary tuberculosis, twenty-seven cases of miliary tuberculosis, tuberculous meningitis, or both in the same patient, and sixteen cases of tuberculous peritonitis. Though resistant coliforms were found, during treatment, in only 8.6 per cent of the cases of nonmiliary pulmonary tuberculosis, they were encountered in 62.9 per cent of the miliary-meningitis group and 62.5 per cent of the peritonitis cases.

*By the Shigella Typing Laboratory of the Communicable Disease Center of the Public Health Service, Chamblee, Ga., through the courtesy of Dr. Martin Frobisher, Jr., Dr. Philip R. Edwards, and Dr. W. H. Ewing.

In the preparation of Table I, a case was included in the "resistant" group if resistant coliforms were found in one or more stool specimens during treatment. In seventeen cases, once the resistant variants had appeared, they were found in all subsequent specimens until several weeks after the antibiotic was discontinued.[4] In seven cases, however, they appeared and disappeared during administration of streptomycin. No stools were obtained from six patients after the initial discovery of resistant variants.

The presence of resistant variants in any specimen was immediately revealed by growth on the streptomycin plate. During the entire study, 213 colonies were picked from these plates and assayed in the serial dilution test. For growth inhibition, 90.1 per cent of the colonies required 625 μg streptomycin per cubic centimeter or more, and 99.8 per cent needed 312 micrograms. In most of the cases where streptomycin-resistant strains were encountered, only resistant colonies were found on the *plain* plates as well, indicating that the original, sensitive strains had been largely or entirely replaced by the resistant variants. Occasionally, however, the plain plate yielded sensitive strains or a mixture of resistant and sensitive variants even when there was growth on the streptomycin plate, demonstrating the concomitant presence of both forms in the same stool specimen. This phenomenon was also noted during the replacement of resistant by sensitive variants after discontinuance of treatment and has been reported elsewhere.[4]

TABLE I. APPEARANCE OF STREPTOMYCIN-RESISTANT COLIFORM BACILLI IN STOOLS DURING STREPTOMYCIN TREATMENT OF VARIOUS TYPES OF TUBERCULOSIS

TYPE OF TUBERCULOSIS	NUMBER OF PATIENTS	PER CENT OF PATIENTS WHERE RESISTANT COLIFORMS WERE FOUND IN 1 OR MORE SPECIMENS
Pulmonary (not miliary)	35	8.6
Miliary and/or meningitis	27	62.9
Abdominal and/or peritoneal	16	62.5

These data have been subjected to the chi square test, as described in Hill, *Principles of Medical Statistics*, London, 1942, pp. 78 to 86. According to this test and Fisher's probability tables, the chances of the difference between the incidence of streptomycin-resistant coliforms in pulmonary tuberculosis and either of the other two types occurring by chance is less than 0.01 per cent.

Several other types of tuberculosis were represented in the study by statistically insignificant numbers of cases. There were six cases of pericarditis, in three of which streptomycin-resistant coliforms were found. They were not found in any of three cases of multiple cold abscesses. On the other hand, they were encountered in two cases of pleurisy with effusion and two cases of Pott's disease.

Time of Appearance of Resistant Variants, During Treatment.—Table II presents the thirty cases where resistant variants were found, according to the week they first appeared. In 16.7 per cent, resistant strains were discovered during the first week of treatment, and in 80 per cent they had appeared by the sixth week, the earliest time when streptomycin was discontinued in this series.

TABLE II. TIME OF APPEARANCE OF RESISTANT COLIFORMS DURING STREPTOMYCIN TREATMENT

WEEK OF TREATMENT RESISTANT COLIFORMS FIRST FOUND	NUMBER OF PATIENTS				CUMULATIVE PERCENTAGE
	PUL.	MIL. MEN.	PER.	TOTAL PATIENTS EACH WEEK	
1	0	3	2	5	16.7
2	1	3	3	7	40
3	1	4	1	6	60
4	0	0	1	1	63.4
5	1	1	0	2	70.2
6	0	3	0	3	80
7	0	2	0	2	86.8
8	0	0	0	0	86.8
Later than 8	-	1	3	4	100

Pul., Nonmiliary pulmonary.
Mil. men., Miliary-meningitis.
Per., Peritonitis.

It is noteworthy that the time of appearance bore no relation to the type of tuberculosis.

Lack of Relation of Streptomycin Dosage to Appearance of Streptomycin-Resistant Coliforms.—Since most of the patients with nonmiliary pulmonary tuberculosis were given 1 Gm. a day, whereas many patients in the other groups received 2 Gm. per day, it seemed possible that the dosage of streptomycin might play a determining role in regard to the appearance of resistant bacteria. That this was actually not the case is shown by the data in Table III. In the miliary-meningitis group, the percentage of patients developing resistant coliforms on 1 Gm. a day was 63.5, compared with 64.4 per cent for patients given 2 Gm. a day. Among the peritonitis patients, 55.5 per cent of patients on 1 Gm. a day and 83.3 per cent of those on 2 Gm. a day displayed resistant coli-

TABLE III. LACK OF RELATION OF STREPTOMYCIN DOSAGE TO APPEARANCE OF STREPTOMYCIN-RESISTANT COLIFORMS

TYPE OF TUBERCULOSIS	STREPTOMYCIN DOSAGE (GM. PER DAY)	
	1.0	2.0
	PER CENT OF PATIENTS	
Miliary-meningitis		
Resistant coliforms	63.5	64.4
No resistant coliforms	36.5	35.6
Total per cent	100	100
Total patients	11	14
Peritonitis		
Resistant coliforms	55.5	83.3
No resistant coliforms	44.5	16.7
Total per cent	100	100
Total patients	9	6
Pulmonary		
Resistant coliforms	6.3	0
No resistant coliforms	93.7	100
Total per cent	100	100
Total patients	32	2

One patient with pulmonary tuberculosis received 0.5 Gm. a day. Resistant *Esch. coli* appeared in this case during the second week of treatment.

forms. In contrast, 6.3 per cent of patients with pulmonary tuberculosis on 1 Gm. a day developed resistant coliforms, but neither of two patients given 2 Gm. a day.

Assay of Streptomycin in Stools.—Since streptomycin-resistant bacteria flourish in culture mediums where the growth of streptomycin-susceptible organisms is suppressed by the antibiotic, it seemed desirable to determine whether there was a difference in the amount of streptomycin present in the stools of representative patients in this series. Assays were made accordingly upon fifty-six specimens from twenty-two patients during streptomycin therapy and upon thirteen specimens from four patients not receiving the drug. No streptomycin was demonstrated in the latter group.

Table IV indicates that within the limits of the quasi-quantitative technique employed there was no significant difference in the amount of streptomycin found in stools exhibiting resistant coliforms and those not yielding resistant variants. The number of specimens did not permit conclusions to be drawn in regard to the amount of the drug present in different clinical types of tuberculosis. Both high and low values were found in pulmonary, miliary, and peritoneal tuberculosis.

TABLE IV. STREPTOMYCIN IN FIFTY-SIX STOOL SPECIMENS FROM PATIENTS RECEIVING THE DRUG INTRAMUSCULARLY

STREPTOMYCIN IN WET STOOL (μg PER GM. STOOL)	PER CENT SPECIMENS SHOWING STREPTOMYCIN-RESISTANT COLIFORMS	PER CENT SPECIMENS SHOWING NO RESISTANT COLIFORMS
6 or less	50.1	53.6
12-100	49.9	46.4
Number of specimens	28	28

Serologic Typing of Streptomycin-Sensitive and Resistant Esch. coli From the Same Patient.—At least one sensitive and one resistant strain from fifteen patients were studied in regard to serologic type.* In Cases 1, 4, 6, 11, and 12, sensitive and resistant variants were obtained from the same stool specimen. In every other case, the sensitive strains were obtained weeks or months before or after the resistant strain was isolated. The results are presented in Table V.

The sensitive and resistant variants proved to be the same type in only two instances (Cases 1 and 2). In four instances (Cases 12, 13, 14, and 15) one or the other variant could not be typed. In every other instance the sensitive and resistant strains differed from one another serologically. Sensitive strains of the same type were recovered twice from each of three patients (Cases 3, 4, and 5), once from the same stool, but in specimens one and four months apart in the other two cases. Two resistant strains of the same type were found in Case 6, but in four other instances where typing was attempted on more than one resistant strain from the same patient (Cases 4, 10, 13, 14) the serologic identity of the colonies could not be established.

In contrast to the findings among the stool coli is the typing of three pairs of pathogenic colon bacilli, where the two members of each pair proved to be

*By the Shigella Typing Laboratory of the Communicable Disease Center, Chamblee, Ga.

serologically identical. In one case, streptomycin-sensitive and resistant colon bacilli from the urine were type 02; in another patient with a urinary tract infection two sensitive colonies of type 06 were found. The third case was one of a blood stream infection presumably from a decubitus ulcer. Coli from the blood and the ulcer were both type 02.

TABLE V. SEROLOGIC TYPING OF ESCH. COLI FROM STOOL SPECIMENS

	SENSITIVE STRAINS			RESISTANT STRAINS		
CASE	STRAIN	DATE OF STOOL SPECIMEN	TYPE	STRAIN	DATE OF STOOL SPECIMEN	TYPE
1	CA 1C CA 5C	11 Apr. 50 25 Apr. 50	034 034	CA 3C	25 Apr. 50	034
2	MS 3C	28 Feb. 49	02	MS 5C	13 Apr. 49	02
3	ML 3C ML 19C ML 18C	3 Aug. 49 12 Oct. 49 12 Oct. 49	09 036 036	ML 6C	23 Aug. 48	02
4	CS 1C CS 7C	5 Apr. 50 3 May 50	02 02	CS 4C CS 5C CS 6C	5 Apr. 50 5 Apr. 50 5 Apr. 50	Rough Rough Rough
5	HR 3C HR 32C	10 Feb. 49 23 June 49	037 037	HR 12C	19 Apr. 49	01
6	AW 8C	13 Apr. 49	062	AW 1C AW 17C	13 Apr. 49 16 Apr. 49	01 or 031 031
7	HH 4C	12 Feb. 48	028	HH 1C	27 Sep. 48	021
8	EW 9C	10 May 49	04	EW 7C	29 May 49	05
9	CE 1C CE 14C	24 Jan. 49 21 Apr. 49	025 (tent.) Untyped	CE 12C	13 Apr. 49	0111:B_4
10	GS 9C	18 May 49	050	GS 4C GS 8C	28 May 49 28 May 49	Untyped 075
11	EK 1C	25 Jan. 49	041	EK 4C	25 Jan. 49	061
12	HS 10C	14 Feb. 49	037	HS 13C	14 Feb. 49	Untyped
13	LP 133C LP 176C	2 May 48 18 May 48	Untyped Untyped	LP 21C LP 81C	24 Feb. 48 5 Apr. 48	Untyped 07
14	RH 9C RH 10C RH 11C RH 12C	5 Oct. 49 5 Oct. 49 18 Jan. 50 18 Jan. 50	Rough Rough Undetermined Related to 076	RH 3C RH 4C	8 Nov. 49 8 Nov. 49	Rough Rough
15	LF 2C	31 Jan. 49	Related to 0.25	LF 5C	3 Aug. 49	Untyped

DISCUSSION

Several authors have noted that streptomycin-resistant tubercle bacilli are found more often in certain clinical types of tuberculosis than in others. Thus, Mitchell[5] encountered resistant tubercle bacilli in 40 per cent of cases with pretreatment evidence of cavity and no coincident collapse, but in only 11 per cent of cases without cavity before treatment. A group of English physicians[6] commented on the apparently greater incidence of appearance of drug-resistant forms in patients who were seriously ill at the beginning of treatment. Though these experiences cannot be directly correlated with our own, they indicate that tissue factors may play a role in the development of resistant bacteria *in vivo*. In our series, the patients with miliary or meningitic tuberculosis were

more seriously ill, as a group, than those with pulmonary tuberculosis, though we could not establish a relationship between degree of clinical illness and development of resistant coliforms in individual cases. On the other hand, our patients with tuberculous peritonitis were not clearly as ill as those in the miliary-meningitis group. Actually these considerations do not lead to a concrete explanation of our results, which must remain unexplained at the present time. It had seemed possible that in the peritoneal cases, and in the seriously ill miliary and meningitic patients, more streptomycin might gain entrance into the bowel than in ordinary pulmonary tuberculosis. This did not prove to be the case, however. Also, neither the daily nor total dosage of streptomycin was important.

The great variation in the serologic types found among sensitive and resistant variants is not surprising, in view of the studies of Kauffmann[3] and others.[7] These investigators have shown that the types of *Esch. coli* present in the stools of normal human beings are constantly changing. It is worthy of comment, therefore, that the resistant variants recovered during streptomycin therapy usually differed serologically from the sensitive strains found in the same patient. The number of colonies that could be typed was far too small to permit broad generalizations. Still, it was interesting that type 02, resistant variants of which were found as commensals in the stools of two tuberculosis patients, was responsible for two of the three urinary or blood stream infections where coli were available for typing.

Finally, the appearance and disappearance of resistant variants during the administration of streptomycin deserves mention, even though its explanation is obscure. There is no evidence that this was due to fluctuation in the concentration of streptomycin in the stool, though this possibility has not been entirely disposed of.

SUMMARY

A study has been made of the streptomycin resistance of coliform bacilli in the stools of patients treated with streptomycin for various clinical types of tuberculosis.

Streptomycin-resistant variants were found in only one of ninety-three stool specimens from patients not receiving streptomycin. The resistant variants were present in very small numbers in this specimen.

Resistant coliforms appeared during treatment in the stools of 62.9 per cent of patients with miliary tuberculosis and/or tuberculous meningitis, 62.5 per cent of patients with tuberculous peritonitis, but only 8.6 per cent of patients with nonmiliary pulmonary tuberculosis. This difference is statistically significant.

In 40 per cent of the cases in which resistant variants were discovered they had appeared by the end of the second week of treatment, and in 80 per cent by the end of the sixth week.

The appearance of resistant variants could not be correlated with dosage of streptomycin or with the concentration of the antibiotic in the stool.

In most instances the serologic type of the resistant strain recovered from any given patient differed from that of the sensitive strain recovered from the same patient. In a few instances, the sensitive and resistant variants were serologically alike.

Resistant variants of type 02 were found as commensals in the stools of two patients with tuberculosis. The same type was also encountered as a pathogen in a urinary tract infection and in a patient with *Esch. coli* septicemia.

REFERENCES

1. Hamburger, Morton, Berman, Jerome R., and Fabrizio, Angelina: The Development of Streptomycin-Resistant Bacteria in the Stools of Patients Treated for Tuberculosis, J. LAB. & CLIN. MED. **33**: 1460, 1948.
2. Parr, L. W.: Sanitary Significance of Succession of Coli-Aerogenes Organisms in Fresh and in Stored Feces, Am. J. Pub. Health **26**: 39, 1936.
3. Kauffmann, F.: Review. The Serology of the Coli Group, J. Immunol. **57**: 71, 1947.
4. Hamburger, Morton, and Berman, Jerome R.: The Replacement of Streptomycin-Resistant Coliform Bacteria in the Stools by Streptomycin-Sensitive Variants During and Following the Cessation of Streptomycin Therapy, J. Clin. Investigation **29**: 630, 1950.
5. Mitchell, Roger S.: The Relation of Cavity to the Development of Streptomycin-Resistant Tubercle Bacilli in Pulmonary Tuberculosis, New England J. Med. **241**: 450, 1949.
6. Streptomycin in Tuberculosis Trials Committee, Medical Research Council Investigation: Streptomycin Treatment of Pulmonary Tuberculosis, Brit. M. J. **2**: 780, 1948.
7. Stuart, C. A., Baker, Muriel, and others: Antigenic Relationships of the Coliform Bacteria, J. Bact. **40**: 101, 1940.

PRIMARY PNEUMOCOCCIC PNEUMONIA AT THE CINCINNATI GENERAL HOSPITAL, 1936-1950

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PRIMARY pneumococcic pneumonia has been a serious public health problem in Cincinnati, Ohio. In the early 1930's there were more than one hundred deaths out of more than three hundred patients admitted to the Cincinnati General Hospital each year with this disease. Now, fourteen years later, there are eight to ten deaths out of approximately one hundred or more patients admitted each year.

The production of pneumococcus typing serum in the 1930's provided a method for the specific identification of the infecting pneumococcus. Early in 1936 typing serum was available for almost all of the pneumococcus types reported here, so that a long-term study of pneumococcic pneumonia was planned.

This study is a review of all of the cases of primary pneumococcic pneumonia admitted to the medical service of the Cincinnati General Hospital from July 1, 1936, to July 1, 1950. There were 3,205 cases. All of these patients were 13 years of age or older. All patients exhibited a typed pneumococcus in sputum or blood, and no distinction was made as to whether the distribution of the infiltrate was lobar or lobular. Only primary pneumonia which developed while the patient was ambulatory, and in ordinary health, is reported here. Many of these patients gave a history of preceding upper respiratory symptoms, but the clinical diagnosis of primary pneumococcic pneumonia was not altered by the presence or absence of this history. Almost all of these patients received no specific treatment prior to admission to the hospital, except for a rare occasional patient in later years who was given sulfonamide or penicillin before hospitalization. Patients with purulent complications due to pneumococcus infection were included if history and findings indicated that the complication resulted from primary pneumococcic pneumonia. These cases were included whether or not the complication was present before the patient was admitted to the hospital. Patients who died within twenty-four hours of admission were included. Since pneumonia occurred most frequently during the winter months, the data for each year are calculated from July 1 through June 30 of the following year.*

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*A preliminary report of this study was presented in abstract at the 1949 meeting of the Central Society for Clinical Research.[1]

METHODS

Plan of Study.—Before specific treatment was administered, samples of sputum, or throat swab, and blood were obtained from each patient for culture. Three cubic centimeters to 5 c.c. of the patient's blood were added to 50 c.c. of beef heart infusion broth, and an agar pour plate was prepared with 1 c.c. of the patient's blood. This method of blood culture was continued uniformly throughout the fourteen years of the study, but the method of examining sputum was gradually modified.

In the earlier years, when type-specific antipneumococcic serum was the only treatment, it was essential to learn the type of the infecting pneumococcus promptly. Therefore sputum was typed directly, or cultured in 3 per cent rabbit's blood broth which contained 1 per cent glucose. Sputum from certain patients who had been ill less than ninety-six hours was injected intraperitoneally into a mouse if the sputum did not type directly. The mouse was sacrificed and the peritoneal exudate was typed directly for pneumococci, or the mouse heart blood was cultured. Analysis of these methods revealed that mouse inoculation was the only method by which a pneumococcus was demonstrated in approximately 12 per cent of the patients in whom a typed pneumococcus was found.

Therefore, approximately 12 per cent of the cases remained unidentified after July 1, 1942, when mouse inoculation was discontinued. An additional small unknown percentage of cases was lost after July 1, 1943, because the pneumonia laboratory was closed for one or two months each summer because of a scarcity of cases.

The number of pneumococci in sputum was not counted. In a few cases, which did not exhibit a pneumococcus in blood or sputum, needle aspiration of lung was performed to demonstrate the type of infecting pneumococcus for the administration of serum therapy. In a few other instances a pneumococcus was demonstrated in the blood culture when it was not found in the sputum.

If two or more types of pneumococcus were demonstrated in the sputum, one type was assigned as the infecting type for the purpose of this study. If bacteremia was present, the typed pneumococcus in the blood culture was taken as the etiological agent. If blood culture was negative the predominating pneumococcus typed in the sputum was taken as the cause of the pneumonia. If two or more types demonstrated in the sputum were few in number, and bacteremia was not present, the lowest type was taken as the etiological agent. Bacteremia due to more than one type of pneumococcus was not observed.

RESULTS

Observations of primary pneumococcic pneumonia, 1936 to 1950, are presented according to the following outline.

A. Mortality per year of patients classified as follows:
   1. All patients, compared with the usual treatment (Table I).
   2. Sex (Table II).
   3. Age (Table III).
   4. Race (Table IV).
   5. Race, according to age (Table V).
   6. Bacteremia (Table VI).
   7. Purulent complication (Table VII).

B. Observations of the pneumococcus types per year.
   1. Per cent frequency of all types (Table VIII).
   2. Number of cases due to the predominating Types (1, 2, 3, and 7), in patients classified as follows (Table IX):
      (a) All patients.
      (b) Deaths.
      (c) Age 60 years and above.
      (d) Bacteremic.
      (e) Purulent complications.

A. Mortality per year.—Table I presents the number of cases of primary pneumococcic pneumonia each year, compared with the usual treatment each year. The number of pneumonia cases per year decreased irregularly from 387 in 1936-37 to 100 in 1949-50. This decline was not due to a decrease in the total number of cases admitted to the medical service per calendar year. Excepting 1936, this total number of cases held steadily between a high of 2,482 in 1939 and a low of 2,117 in 1946. The pneumonia mortality decreased irregularly from the high of 36.7 per cent in 1936-37 to the low of 6.5 per cent in 1948-49.

The "usual" treatment, as listed in Table I, was adhered to in uncomplicated cases if response to treatment was satisfactory. However, if a complication was present or response to treatment was unsatisfactory, dosages were increased and therapeutic agents were used in combination when it was considered necessary for the good of the patient. Therefore this information merely demonstrated four eras of therapy: that of serum, sulfonamide, sulfonamide with adjuvant penicillin, and penicillin. In 1949-50 aureomycin was used for the treatment in alternate cases, and these results will be presented in a subsequent report. For present purposes, however, the aureomycin-treated patients are included in the three-year penicillin era. In summary then, the era of serum treatment (1936-37 through 1938-39) showed a 32.5 per cent mortality, the era of sulfonamide treatment (1939-40 through 1944-45) a 16.9 per cent mortality, the era of sulfonamide with adjuvant penicillin treatment (1945-46 and 1946-47) a 13.0 per cent mortality, and the era of penicillin treatment (1947-48 through 1949-50) an 8.6 per cent mortality.

Table II presents the mortality according to sex. Although 69.4 per cent of all patients were male, there was no difference between the mortality of the male and female patients.

Table III presents the mortality according to the younger, middle, and older age groups; 43.5 per cent of all patients were 13 to 39 years of age, 35.4 per cent were 40 to 59 years of age, and 21.0 per cent were 60 years of age and older.

In all treatment eras, excepting the era of penicillin treatment (1947-48 through 1949-50), there was a definite relationship between the age group and mortality. In the sulfonamide treatment era for example (1939-40 through 1944-45), the mortality per year in the younger age group was 2.4 to 8.5 per cent; in the middle age group it was 12.0 to 23.9 per cent; and in the older age group it was 26.4 to 53.8 per cent . This relationship was less consistent in the penicillin treatment era. In 1947-48 mortality in the middle age group was less than that of the younger age group; and in 1948-49 mortality in the older age group was less than that of the middle age group.

Table IV presents the mortality according to race. 54.1 per cent of all patients were white. Excepting 1939-40, a consistently higher percentage of white patients died each year. In the sulfonamide treatment era for example (1939-40 through 1944-45), the mortality per year of white patients was 14.2 to 27.0 per cent and the mortality of Negro patients was 9.2 to 17.7 per cent. This relationship held true for the other treatment eras also. However this

TABLE I. FREQUENCY, MORTALITY, AND THE USUAL TREATMENT PER YEAR IN PRIMARY PNEUMOCOCCIC PNEUMONIA AT THE CINCINNATI GENERAL HOSPITAL, 1936-1950

	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
Number of pneumonia cases	387	281	359	375	287	242	215	221	167	194	151	104	122	100
Per cent died	36.7	32.4	28.1	15.2	20.2	13.6	15.3	19.5	18.6	13.4	12.6	9.6	6.5	10.0
Usual treatment	Serum	Serum	Serum	SPD Serum	SPD STZ SPZ	SPZ STZ	STZ	SDZ STZ SMZ	SMZ SDZ	SMZ* SDZ PNC	SMZ* SDZ PNC	PNC†	PNC†	PNC‡ AMC

SPD, sulfapyridine; STZ, sulfathiazole; SPZ, Sulfapyrazine; SDZ, Sulfadiazine; SMZ, Sulfamerazine; PNC, penicillin; AMC, aureomycin.

*In 1945-46 and 1946-47 intramuscular amorphous penicillin in dosage of 25,000 units every three hours was prescribed only if the patient did not respond to sulfonamide, or if sulfonamide was contraindicated.

†In 1947-48 and 1948-49 intramuscular crystalline penicillin dosage was 200,000 units or 300,000 units once daily plus one additional dose given twelve hours after the initial dose.

‡In 1949-50 certain patients were given aureomycin. The dosage was 0.5 Gm. every six hours. Results of this treatment will be presented in a subsequent report.

TABLE II. MORTALITY ACCORDING TO SEX*

	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
Mortality (per cent)														
Male	38.3	33.8	28.6	14.1	21.0	12.4	17.2	19.9	22.1	13.1	15.4	9.6	7.7	8.5
Female	34.3	29.1	25.0	17.6	20.6	19.3	17.3	20.0	11.1	15.6	17.0	9.7	3.2	13.3

*69.4 per cent of all patients were men. The frequency of male patients per year ranged between 64.3 per cent in 1938-39 and 76.4 per cent in 1941-42.

TABLE III. MORTALITY ACCORDING TO AGE*

	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
Mortality (percent)														
Age 13 to 39	18.2	17.0	12.3	7.2	8.5	6.0	5.9	2.4	6.7	1.2	1.8	2.3	2.0	0
Age 40 to 59	47.4	38.3	32.2	15.8	23.1	18.0	12.0	23.9	16.9	15.9	15.4	0	10.9	9.5
Age 60 and above	58.5	56.8	61.6	32.5	43.3	26.4	53.8	43.2	38.0	32.6	34.0	30.0	8.0	24.0

*43.5 per cent of all patients were 13 to 39 years of age, 35.4 per cent were 40 to 59, and 21.0 per cent were 60 and older. The frequency of the 13 to 39 group per year ranged between 33.0 per cent in 1949-50 and 49.6 per cent in 1938-39; the 40 to 59 group ranged between 29.7 per cent in 1941-42 and 42.0 per cent in 1949-50; the 60 and older group ranged between 16.7 per cent in 1938-39 and 29.1 per cent in 1946-47.

TABLE IV. MORTALITY ACCORDING TO RACE*

	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
Mortality (per cent)														
White patients died	39.2	37.4	30.6	14.2	23.3	18.2	25.7	27.0	26.4	16.7	20.5	15.2	8.0	15.6
Negro patients died	34.4	24.5	21.6	16.9	17.7	10.6	9.9	11.1	9.2	10.9	11.5	5.2	5.5	7.3

*54.1 per cent of all patients were white. The frequency of white patients per year ranged between 32.0 per cent in 1949-50 and 62.6 per cent in 1938-39.

difference was demonstrated to be due primarily to a difference in the age distribution of the races. A notable majority of the patients above 60 years of age were white.

Table V presents the mortality according to race in patients above and below 60 years of age. A total of 674 patients were sixty years of age or older, and 74.7 per cent of these were white. Two thousand five hundred thirty-one patients were 13 to 59 years of age, and only 48.9 per cent of these were white. No consistent difference of the mortality per year according to race in these two age groups was demonstrated. The rather marked fluctuation of yearly mortality of patients in the older age group is probably due to the small number of cases each year.

Table VI presents the mortality according to bacteremia. A typed pneumococcus in blood culture was exhibited by 22.8 per cent of all patients. Excepting 1947-48, 16.4 to 31.8 per cent of patients each year had bacteremia. In 1947-48, 49.0 per cent had bacteremia. Mortality was much higher in bacteremic patients than in patients with negative blood culture. In the sulfonamide treatment era for example (1939-40 through 1944-45), the mortality of bacteremic patients per year was 32.3 to 56.7 per cent and the mortality of nonbacteremic patients was 7.0 to 14.8 per cent. The same relationship held true in the other treatment eras.

Table VII presents the mortality according to purulent complication. Purulent complications were exhibited by 7.1 per cent of all patients. In Table VII no differentiation is made as to whether the complications developed before or after treatment was started.*

An unexpected observation is that the per cent of cases each year with purulent complications and the mortality of these complications were essentially the same in all eras of treatment. Purulent complications occurred in 3.5 to 12.4 per cent of cases each year. The mortality of these patients per year was 25.0 to 87.0 per cent, whereas that of patients without purulent complications was 4.3 to 33.2 per cent.

B. Observations of the Pneumococcus Types Per Year.—Table VIII presents the per cent of cases each year which were caused by each of the pneumococcus types. Types 1, 2, 3, and 7 were the predominating types. Pneumonia due to these types comprised approximately 40 to 50 per cent of the cases each year. Type 1 was most frequent in eight years and Type 7 most frequent in three years (1941-42, 1945-46, and 1946-47). Types 1 and 7 were equally frequent one year (1944-45), and Types 3 and 2 were each most frequent one year (1937-38 and 1947-48). Marked fluctuations of the frequency of various pneumococcus types per year were observed. The rise of Type 7 in 1941-42 and the rise of Type 2 simultaneous with the fall of Type 1 in 1947-48 followed by the rise of Type 1 in 1948-49 are notable examples.

Table IX is a composite table which presents the number of cases in various classifications each year which were due to each of the predominating pneumococcus types (1, 2, 3, and 7). The total number of cases due to each of these types each year is presented here for comparison with the other data.

*These complications were endocarditis, meningitis, empyema, lung abscess, and pyarthrosis. An analysis of these complications will be presented in a separate report.

TABLE V. MORTALITY ACCORDING TO RACE IN PATIENTS ABOVE AND BELOW 60 YEARS OF AGE

	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
	AGED 60 AND ABOVE*													
Number of cases	70	58	60	80	53	53	39	46	42	49	44	30	25	25
Mortality (per cent)														
White	55.5	57.4	60.0	27.8	50.0	25.5	54.8	45.9	41.0	32.5	48.1	43.7	7.1	25.0
Negro	64.0	54.5	70.0	47.3	18.1	30.0	50.0	33.3	16.6	33.3	11.7	14.2	9.0	23.0
	AGED 13 TO 59†													
Number of cases	317	223	299	295	234	189	176	175	125	145	107	74	97	75
Mortality (per cent)														
White	33.5	30.5	21.1	9.3	14.1	13.0	14.4	17.5	15.7	5.1	4.2	0	6.5	10.0
Negro	30.7	20.5	17.7	12.1	17.5	9.1	5.6	9.5	8.8	9.1	11.6	2.3	5.8	3.6

*A total of 674 patients were 60 years of age or older; 74.7 per cent of these were white. The frequency of white patients per year in this age group ranged between 44.0 per cent in 1949-50 and 87.7 per cent in 1945-46.

†A total of 2,531 patients were 13 to 59 years of age; 48.9 per cent of these were white. The frequency of white patients per year in this age group ranged between 26.6 per cent in 1949-50 and 58.5 per cent in 1938-39.

TABLE VI. MORTALITY ACCORDING TO BACTEREMIA

	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
Per cent of patients with bacteremia	31.8	17.8	16.4	17.3	12.9	30.2	27.4	20.8	20.4	17.5	30.5	49.0	26.2	23.0
Mortality (per cent)														
With bacteremia	69.9	70.0	47.4	32.3	56.7	37.0	37.3	56.5	35.3	38.2	28.3	13.7	21.9	17.4
Without bacteremia	21.2	24.2	24.3	11.6	14.8	3.5	7.0	9.7	14.3	8.1	5.7	5.7	1.1	7.7

TABLE VII. MORTALITY ACCORDING TO PURULENT COMPLICATION*

	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
Per cent of patients with purulent complication	8.2	12.4	5.5	5.6	3.5	6.6	10.2	9.9	7.2	5.1	5.2	8.6	4.9	8.0
Mortality (per cent)														
Purulent complication	75.0	77.0	65.0	45.0	70.0	87.0	72.0	72.0	25.0	50.0	50.0	33.3	50.0	62.5
No purulent complication	33.2	26.0	25.9	13.5	18.4	8.4	8.6	13.6	18.0	11.4	10.5	7.4	4.3	5.4

*These complications were endocarditis, meningitis, empyema, lung abscess, and pyarthrosis.

TABLE VIII. FREQUENCY OF PNEUMOCOCCUS TYPES PER YEAR IN PRIMARY PNEUMOCOCCIC PNEUMONIA AT THE CINCINNATI GENERAL HOSPITAL, 1936-1950*

TYPES	PER CENT OF CASES EACH YEAR WHICH WERE CAUSED BY THE GIVEN PNEUMOCOCCUS TYPE													
	YEAR													
	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
1	*18.6*	11.4	*13.6*	*16.3*	*12.9*	12.4	*13.5*	*21.7*	*16.2*	11.8	19.2	6.7	*20.5*	*16.0*
2	12.1	11.7	12.8	9.9	8.7	12.4	10.2	7.7	5.4	1.5	2.6	*16.5*	9.0	4.0
3	14.5	*14.6*	13.1	15.5	11.8	12.4	10.7	7.7	5.4	10.8	6.6	6.7	6.6	10.0
4	4.4	5.7	7.0	5.3	4.2	4.9	6.5	5.9	5.4	4.1	2.6	5.8	4.9	5.0
5	8.0	5.3	6.7	4.8	4.9	8.3	4.2	3.2	8.4	4.6	4.6	4.8	12.3	5.0
6	1.8	3.2	3.9	3.2	5.9	3.3	2.3	0.9	1.2	3.1	3.3	2.9	0	3.0
7	10.6	11.4	7.2	8.3	8.0	*18.6*	12.1	17.6	*16.2*	*17.5*	*19.9*	13.5	14.7	8.0
8	7.0	7.8	7.8	4.8	9.4	4.1	7.4	3.6	2.4	4.1	9.9	4.8	4.1	11.0
9	2.0	4.3	2.5	1.9	0.7	1.6	1.4	0.9	1.8	3.6	1.3	1.9	3.3	3.0
10	1.5	2.5	1.4	1.3	4.2	1.6	0.9	1.8	1.8	2.6	1.3	1.9	0.8	4.0
11	2.6	1.8	2.8	1.1	1.0	0.4	1.4	0.9	0.6	0	0.7	1.0	2.4	0
12	2.0	1.8	1.4	1.6	1.7	2.1	4.6	4.1	4.2	1.5	2.6	3.8	6.6	2.0
13	0.2	1.4	0.5	1.9	1.0	1.6	1.4	3.2	1.2	2.6	1.3	1.0	0.8	3.0
14	1.0	0.7	1.7	2.4	3.1	2.1	4.2	1.3	1.8	2.6	4.0	6.7	3.3	4.0
15	0.2	1.8	0.3	1.3	0	0	0	2.3	2.4	1.5	0.7	1.0	1.6	1.0
16	0.5	0.3	0.8	1.3	2.8	1.2	1.4	0	1.8	0.5	0.7	0	0	1.0
17	1.3	2.5	1.9	1.6	1.4	0.4	1.9	0.4	0.6	1.0	1.3	1.9	1.6	2.0
18	2.3	2.1	1.1	2.7	0.7	1.6	2.8	1.3	3.0	1.5	0.7	2.9	1.6	4.0
19	2.8	3.2	3.0	1.9	0.7	2.5	2.8	0.9	1.8	3.6	2.0	1.9	0.8	3.0
20	1.3	1.1	2.8	2.9	4.9	1.6	0.9	1.3	3.6	3.6	2.0	2.9	0.8	2.0
21	0	0	1.4	0.5	1.4	0.8	0	2.7	1.8	3.6	0.7	0	0	1.0
22	0.2	1.1	1.4	1.3	1.4	0.4	0.9	0.4	1.8	0	0.7	2.9	0	0
23	0.2	0.7	0.8	1.9	1.7	0	0.5	0.4	0	0.5	0	1.0	0	1.0
24	1.0	0.3	1.1	1.3	0.7	0.8	0	1.3	1.2	3.6	2.6	1.0	0	0
25	1.8	1.8	0.5	1.3	0	0.4	3.7	3.2	3.6	2.6	4.0	1.0	0	0
27	0	0.3	0	0.8	0.3	0.4	0.5	0.4	0	0	0.7	0	0	0
28	0.2	0	0.5	0	0.3	0.4	1.4	0	1.2	0	0.7	1.9	0.8	0
29	0.5	0.3	0.8	0.3	1.4	0.4	0.5	0.4	1.2	2.6	0	0	0.8	1.0
31	0.8	0.7	0.5	0.8	0.3	1.2	0	0	0.6	0.5	0.7	1.9	1.6	1.0
32	0	0	0.3	0.8	1.7	0.4	0	0.4	0.6	0	0	1.0	0	0
33	0	0	0	0.8	1.4	1.2	0.9	3.2	1.2	3.6	0.7	0	0	5.0
34	0	0	0	0.3	1.0	0	0.9	0.4	1.8	0.5	2.0	1.0	0.8	0
Total cases	387	281	359	375	287	242	215	221	167	194	151	104	122	100

*36-37 = From July 1, 1936, to June 30, 1937; 37-38 = from July 1, 1937, to June 30, 1938, etc.
The most frequent type in each year is in italics; Types 1 and 7 were equally frequent in 1944-45.

TABLE IX. OBSERVATIONS OF THE PREDOMINATING TYPES (1, 2, 3 AND 7) IN PRIMARY PNEUMOCOCCIC PNEUMONIA AT THE CINCINNATI GENERAL HOSPITAL, 1936-50

(The data are presented as the number of cases for each classification. The greatest number in each year is in italics or is noted in a footnote)

YEAR	36-37	37-38	38-39	39-40	40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49	49-50
All Cases														
Type 1	*72*	32	*49*	*61*	*37*	30	*29*	*48*	*27*	23	29	7	*25*	*16*
Type 2	47	33	46	37	25	30	22	17	9	3	4	*17*	11	4
Type 3	56	*41*	47	58	34	30	23	17	9	21	10	7	8	10
Type 7	41	32	26	31	23	*45*	26	39	*27*	*34*	*30*	14	18	8
Others	171	143	191	188	168	107	115	100	95	113	78	59	60	62
Deaths														
Type 1	21	6	6	4	5	5	1	*10*	6	0	1*	0	*2*	0
Type 2	20	11	9	2	4	2	3	1	1	0	0	*2*	0	*1*
Type 3	*30*	*19*	*20*	*21*	*9*	*7*	*7*	7	5	3	1	*2*	0	*1*
Type 7	9	8	8	4	5	5	1	*10*	*7*	*5*	2	1	1	*1*
Others	62	47	58	28	35	14	21	15	12	18	15	5	5	7
Age 60+														
Type 1	6	1	2	2	5	5	2	4	*6*	3	2	0	2†	2
Type 2	1	4	5	2	5	3	3	2	0	0	0	4	1	0
Type 3	*17*	*12*	*15*	*26*	*11*	*16*	*8*	3	4	*9*	4	*6*	3	*4*
Type 7	8	4	5	5	4	4	2	*7*	4	4	*8*	4	4	0
Others	38	37	33	45	28	25	24	30	28	33	30	16	15	19
Bacteremia														
Type 1	*25*	1	9	*16*	*8*	*15*	*10*	*19*	*10*	*8*	8‡	4	5‡	*6*
Type 2	20	*12*	*14*	7	6	11	7	3	1	1	1	*13*	2	2
Type 3	16	8	8	13	3	7	3	4	0	4	2	3	2	1
Type 7	13	5	4	6	6	14	6	9	7	6	10	9	4	4
Others	49	24	24	23	14	26	33	11	16	15	25	22	19	10
Purulent Complications														
Type 1	*7*	2	*6*	4	1	1	3§	*5*	*4*	1	*2*	1§	1§	0
Type 2	5	*9*	2	2	2	2	3	3	2	0	0	0	1	*1*
Type 3	4	2	1	*5*	*3*	*5*	1	2	0	*2*	0	1	0	*1*
Type 7	5	4	0	3	0	4	0	*5*	2	*2*	*2*	1	0	*1*
Others	13	20	11	6	5	4	15	7	6	4	5	6	5	5

*In 1946-47 three died out of fifteen cases due to Type 8.

†In 1948-49 five out of fifteen cases due to Type 5 were 60 years of age or older.

‡In 1946-47 eleven out of fifteen cases due to Type 8 had bacteremia.
In 1948-49 eleven out of fifteen cases due to Type 5 had bacteremia.

§In 1942-43 five out of sixteen cases due to Type 8 had purulent complications.
In 1947-48 two out of four cases and in 1948-49 two out of eight cases due to Type 12 had purulent complications.

The number of deaths each year due to these types is notable. Type 3 caused the most deaths each year of the first seven years of the study, 1936-37 through 1942-43, even though it was the most frequent type in only one of these years (1937-38). In the next three years, 1943-44 through 1945-46, Type 3 lost much of its virulence and Type 7 attained a notable virulence. In 1943-44, Types 1 and 7 caused an equal number of deaths, and in 1944-45 and 1945-46 Type 7 caused the most deaths. In 1946-47 Type 8 caused the most deaths, with three deaths out of fifteen cases. In the last three years of the study, 1947-48 through 1949-50, no particular type was notable for the mortality it caused. Types 2 and 3 each caused two deaths in 1947-48. Type 2 caused two deaths in 1948-49, and in 1949-50 one death was caused by each of ten different pneumococcus types.

Type 3 led as cause of pneumonia in patients 60 years of age and older in each of the first seven years of study (1936-37 through 1942-43). In the last seven years (1943-44 through 1949-50) Type 3 pneumonia became less frequent, and the type led in only three years as cause of pneumonia in the aged. Type 7 led in two years (1943-44 and 1946-47), Type 1 in one year (1944-45), and Type 5 in one year (1948-49).

Bacteremia was produced most frequently by Type 1 in each of nine years, by Type 2 in three years, and by Types 5 and 8 in one year each. The frequency of bacteremia in 1947-48 was 49 per cent (Table VI). In that year thirteen out of seventeen patients with cases due to Type 2 pneumococcus had that organism in blood culture.

Purulent complications were produced most frequently by Type 1 in each of three years, by Type 3 in three years, by Type 12 in two years, and by Types 2 and 8 one year each. Types 1 and 7 were equally frequent as cause of complications in two years (1943-44 and 1946-47), and Types 3 and 7 in one year (1945-46). In 1949-50 one purulent complication was caused by each of eight pneumococcus types. The per cent of cases each year with purulent complications did not change notably from year to year (Table VII), but on several occasions a pneumococcus type has exhibited an unusual tendency to produce complications. These complications were produced by Type 2 in nine out of thirty-three cases in 1937-38, by Type 8 in five out of sixteen cases in 1942-43, by Type 12 in two out of four cases in 1947-48, and by Type 12 in two out of eight cases in 1948-49.

DISCUSSION

Two marked decreases of mortality of primary pneumococcic pneumonia occurred with the introduction of more effective therapeutic agents. Mortality was approximately halved by the effective use of sulfonamides (1939-40) and again approximately halved by the exclusive use of penicillin therapy (1947-48). It is interesting to speculate as to what effect these therapeutic agents may have had upon the frequency of pneumococcic pneumonia.

Observations reported here give no clue as to why the frequency of this pneumonia decreased. Several possibilities exist. First, it is possible that more and more patients were treated for pneumonia at home and these cases

were not identified specifically as pneumococcic pneumonia. With the shortage of physicians in Cincinnati during the war, 1941-42 through 1944-45, this possibility seems remote. Another possibility is that the infectivity of pneumococci in the endemic flora was gradually attenuated by effective treatment of the acute infections. A third possibility is that more and more cases of pneumonia were due to pneumococcus types above Type 34, and therefore were not identified in this study. Although the cause is not clear, there has been a definite decrease in the frequency of pneumococcic pneumonia at this hospital, with an increasing number of nonpneumococcic or "virus type" pneumonias. Unfortunately, it is impossible to state which or how many of the cases of primary pneumococcic pneumonia reported here were preceded by a virus infection.*

Three other long-term reviews of pneumococcic pneumonia have been published recently.[4, 5, 6] Brown and Lockhart,[4] in the only other report of consecutive observations of pneumococcus types, also report pronounced fluctuations in the frequency of various types over the years. Their data, from the San Francisco Hospital, also record an increase in the frequency and the mortality of Type 7 pneumonia, which occurred in the three years 1942-43 through 1944-45. Maclachlan and co-workers[5] report a mortality of pneumococcic pneumonia from the Mercy Hospital, Pittsburgh, Pa., which is comparable with the mortality reported here. Likewise they report a 40 per cent frequency of bacteremia in January, February, and March, 1948. This finding is comparable with the occurrence of bacteremia in 49 per cent of cases in the year 1947-48 reported here.

Israel and associates,[6] in a report of pneumococcic pneumonia at the Philadelphia General Hospital, drew their conclusions from an analysis of data taken from the three years 1936-37, 1940-41, and 1945-46. The data and conclusions of their report would be fairly comparable with those reported here if 1936-37, 1940-41, and 1945-46 were the only years included in the present study. However, this method of analysis gives a biased impression, particularly as to the effect of penicillin therapy and the frequency of purulent complications.

Some comment upon the virulence of Type 3 pneumococcus in the first seven years of the study (1936-37 through 1942-43) seems appropriate. In this period Type 3 caused the greatest number of deaths each year and the most cases of pneumonia in the older age group each year, and yet produced relatively few cases with bacteremia. In fact, this type caused almost twice as many deaths, and almost twice as many cases of pneumonia in the aged as it caused bacteremic cases in these seven years. This tendency of Type 3 to infect elderly patients seems to account in a large measure for its record of virulence, in spite of its relatively infrequent invasion of patient's blood. The data of the last seven years (1943-44 through 1949-50) reinforce this concept. While becoming less frequent, Type 3 led in only three years as cause of pneumonia in the aged and did not lead as cause of death.

*The relation of influenza epidemics in Ohio to these cases of primary pneumococcic pneumonia has been reported briefly in abstract[3] and will be reported later in greater detail.

In contrast to Type 3, the increased virulence of Type 7 in the three years 1943-44 through 1945-46 did not depend as much upon an increased tendency to infect elderly patients. In this three-year period Type 7 caused fifteen cases of pneumonia in patients 60 years of age or older, twenty-two deaths, and twenty-two bacteremic cases. The increased virulence of Type 7 apparently depended greatly upon its invasion of the blood stream, often with purulent complications; and sulfonamide therapy was frequently unable to check this infection. In the two years, 1943-44 and 1944-45, when penicillin was not available, eleven of sixteen patients with Type 7 bacteremia died. Five of these patients with fatal cases had meningitis, and two others had lung abscess. When penicillin became available as adjuvant to the sulfonamides in the fall of 1945, Type 7 bacteremia became one of the prime indications for penicillin therapy. Thus, in 1945-46 only two patients with Type 7 bacteremia died. One of these expired a few hours after admission. The other was 75 years old and died two days after admission in spite of penicillin therapy.

Differentiation of the primary pneumococcic pneumonia reported here into lobar and lobular pneumonia was not attempted. If sulfonamide or penicillin treatment was effective the infiltrate was frequently arrested in the lobular phase, before a lobar distribution was attained. Therefore, this anatomic classification of pneumococcic pneumonia lost its meaning with the advent of sulfonamide therapy.

SUMMARY AND CONCLUSION

This study is a review of 3,205 patients with primary pneumococcic pneumonia admitted to the Cincinnati General Hospital in a period of fourteen years, 1936-1950. Of these patients, 43.5 per cent were 13 to 39 years of age, 35.4 per cent were 40 to 59 years of age, and 21.0 per cent were 60 years of age or older.

The number of cases per year decreased irregularly from 387 in 1936-37 to 100 in 1949-50.

Each new era of treatment produced a definite reduction of mortality. The mortality was 32.5 per cent during the three-year era of serum treatment, 1936-37 through 1938-39; 16.9 per cent during the six-year era of sulfonamide treatment, 1939-40 through 1944-45; 13.0 per cent during the two-year era of sulfonamide and adjuvant penicillin treatment, 1945-46 and 1946-47; and 8.6 per cent during the three-year era of penicillin treatment, 1947-48 through 1949-50. The mortality of the older patients was greater than that of the middle-aged or younger patients.

Observations of the predominating pneumococcus types revealed that Type 3 caused the greatest number of deaths in each of the first seven years of the study (1936-37 through 1942-43), and at the same time caused the most cases of pneumonia in the older age group. In the last seven years (1943-44 through 1949-50) Type 3 failed to lead as cause of death and led in only three years as cause of pneumonia in the aged. Type 7 became the most virulent type in the three years of 1943-44 through 1945-46. Type 1 and Type 7 caused an

equal number of deaths in 1943-44, and Type 7 led as cause of death in 1944-45 and 1945-46. Type 8 caused three deaths in 1946-47; and in the last three years, 1947-48 through 1949-50, no type was outstanding as a cause of death.

There was a yearly variation in the frequency of pneumococcus types. Type 1 was the most frequent pneumococcus in eight years; Type 7 was most frequent in three years; Types 1 and 7 were equally frequent one year; and Types 2 and 3 were most frequent one year each.

White patients showed a consistently greater mortality than Negro patients, except for one year, 1939-40. However this was largely due to the large proportion of white patients in the older age group. Of the patients 60 years of age or older, 74.7 per cent were white, whereas only 48.9 per cent of the patients 13 to 59 years of age were white.

The various modes of therapy, which were effective in reducing mortality, had no effect upon the yearly frequency of pneumococcus bacteremia or purulent complications. Bacteremia occurred in 22.8 per cent, and purulent complications occurred in 7.1 per cent of the cases.

REFERENCES

1. Thompson, R. T., Ruegsegger, J. M., Blankenhorn, M. A., and Hamburger, M.: Pneumococcus Types, Mortality, Bacteremia, and Purulent Complications in Primary Pneumococcic Pneumonia at the Cincinnati General Hospital, 1936-1949, J. LAB. & CLIN. MED. **34**: 1757, 1949.
2. Hamburger, M., Berman, J. R., Thompson, R. T., and Blankenhorn, M. A.: The Treatment of Pneumococcal Pneumonia by Penicillin in Aqueous Solution at Long Intervals, J. LAB. & CLIN. MED. **34**: 59, 1949.
3. Thompson, R. T., and Hamburger, M.: The Relation of Influenza Epidemic in Ohio to Primary Pneumococcic Pneumonia at the Cincinnati General Hospital, 1936-1949, J. Clin. Investigation **29**: 849, 1950.
4. Brown, J. W., and Lockhart, J. C.: Pneumococcic Infections in Adults at the San Francisco Hospital from 1932 to 1946, Amer. Pract. **4**: 214, 1949.
5. Maclachlan, W. W. G., Braken, M. N., and Bailey, W. R., Jr.: The Prognosis of Pneumonia, Tr. A. Am. Physicians **61**: 184, 1948.
6. Israel, H. L., Mitterling, R. C., and Flippin, H. F.: Pneumonia at Philadelphia General Hospital, 1936-1946, New England J. Med. **238**: 205, 1948.

INFLUENZA VIRAL INFECTIONS IN BOSTON, SEPTEMBER 1947 TO AUGUST 1949

A Study of the Serologic Response of Patients and of the Antigenic Differences Among the Influenza Viruses That Were Isolated

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PREVIOUS studies made in this laboratory during three separate outbreaks of influenza that occurred in Boston have yielded evidence of the occurrence of more than one antigenically distinct strain of influenza virus during each of these outbreaks.[1-4] Some of the cases of influenza observed in February and March of 1944 were atypical with respect to both the viruses isolated and the antibody responses; no rises in titer of antibodies were demonstrated in serums of a few of these patients for either their own strain or the standard PR8 strain of influenza A and Lee strain of influenza B. The patients observed during the preceding weeks, while the epidemic was at its height, yielded strains which were antigenically similar to the PR8 strain, and antibodies developed in their serums against the latter strain.

In December, 1945, strains of influenza B virus were isolated during the height of the epidemic, but serologic tests indicated the prevalence of influenza A infection for a few weeks thereafter.[2] Furthermore, two antigenically distinct strains of influenza B virus were distinguished among the strains isolated during the height of that epidemic in December; one of these strains appeared to be closely related to the Lee strain and the other was more closely related to the BON strain.[3]

The strains isolated from patients during the epidemic of influenza which occurred in Boston in March and April, 1947, were shown to be antigenically distinct from several "classical" strains of influenza A and from strains isolated during or shortly after the 1943-1944 outbreak.[4] Evidence was obtained which suggested that at least two antigenically distinct strains were active during this 1947 epidemic, one of them closely resembled the FM_1 strain isolated elsewhere* during the same season. In addition, a strain isolated from a sporadic case in December, 1947, showed no apparent antigenic relationship to any of the influenza viruses that were studied.[4] In the latter case, as in some of those observed in March and April, 1944,[1] the patient from whom the strain was isolated failed to develop antibodies in his serum against either his own strain or the standard PR8 and Lee strains of influenza A and B respectively.[4]

The present paper deals with the studies on influenza viral infection carried out in this laboratory over a two-year period from September, 1947, through August, 1949. These include serologic tests in a number of patients with clinical

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influenza and other acute respiratory infections and attempts to isolate influenza viruses from some of these patients. A study of the antigenic relationship among the strains isolated during this period and some standard strains of influenza virus as well as some other strains previously isolated in this laboratory is also included.

MATERIALS AND METHODS

Patients.—Sera for influenzal antibody studies were obtained from various types of cases, including (1) patients with "clinical influenza," (2) some patients with pneumonia and other acute respiratory infections of diverse etiology who were being studied intensively for other purposes, and (3) patients with ill-defined acute respiratory disease. Only those from whom both acute and convalescent serums were obtained about a week or more apart are included, except for a few patients in whom the initial observation was made more than one week after the onset of illness and from whom additional serums were obtained later. The great majority of the patients were from the adult medical wards of the Boston City Hospital, but a few were adults under observation by other physicians in this vicinity.

Viral Isolations.—Influenza viral isolations were attempted principally with throat washings obtained from patients while they were still acutely ill with fever and symptoms of clinical influenza or pneumonia. In a few instances, however, fresh tissues obtained at necropsy from patients who died of fulminating acute respiratory infections were studied. The methods used with throat washings were similar to those described in previous studies.[3] Portions of lungs were finely minced, homogenized in a Waring Blendor, and then used as a 20 per cent suspension in serum broth and then treated like throat washings. At least two amniotic passages in embryonated eggs were made before any material was considered as negative for influenza virus, but multiple attempts were made from the original material in many instances, and larger numbers of blind passages were usually carried out with these specimens. When a virus was isolated, it was passaged twice by the amniotic route and then sixteen or seventeen times allantoically before it was used in serologic studies or for immunization of animals. The isolations were repeated from the original washings in some of these cases.

Serologic Tests.—The hemagglutination inhibition and complement fixation tests were done by methods similar to those used in previous studies.[1] *In ovo* neutralization tests were done by the allantoic route as described by Hirst.[5] Rabbit antiserums for the study of antigenic differences were prepared as in previous studies.[4] Calculations of 50 per cent end points were made by the method of Reed and Muench.[6] All titers are expressed as reciprocals of the initial dilution of serum.

The viral strains used for the studies on antigenic relationships included, in addition to those isolated in the course of the present study, the well-known PR8, Weiss, Swine, and FM_1 strains which may be included among influenza A viruses, the Lee and BON strains of influenza B, strains EB and DW which are representative of the two varieties of virus related to influenza A isolated in March, 1947,[4] and strains MB and MF which are representative of the two varieties of influenza B isolated in December, 1945.[3]

RESULTS

Results of Serologic Tests in Patients: Antihemagglutinins Against the PR8 and Lee Strains.—The results obtained over the two-year period are shown by months in Table I. A total of 383 patients were studied over this period. Significant (fourfold or greater) rises in titer against one of the test strains but not against the other were obtained in the serums of forty-one of these patients—in thirty-three against the PR8 strain of influenza A and in eight against the Lee strain of influenza B virus. All but seven of the thirty-three patients who developed antibodies for influenza A became ill either during March, 1949,

TABLE I. SUMMARY OF RESULTS OF INFLUENZA VIRAL STUDIES IN PATIENTS OBSERVED IN BOSTON, SEPTEMBER, 1947, THROUGH AUGUST, 1949

MONTH	YEAR	RESULTS OF TESTS FOR ANTIHEMAGGLUTININS						VIRUS ISOLATIONS		
		NUMBER OF PATIENTS TESTED	SIGNIFICANT RISES IN TITER			INITIAL HIGH TITER AND TWOFOLD CHANGE		NUMBER ATTEMPTED†	VIRUS ISOLATED‡	
			PR8 ONLY	LEE ONLY	PR8 & LEE*	PR8	LEE		RELATED TO INFLUENZA A	RELATED TO INFLUENZA B
Sept.	1947	13	1	0	2^{2}	0	0	0		
Oct.		33	1	1	3^{2}	0	0	0		
Nov.		11	0	0	2^{2}	0	0	0(+1)		
Dec.		8	0	1	1^{1}	0	0	1	0	1^{1}
Jan.	1948	9	0	0	0	0	0	1	0	0
Feb.		11	1	0	3	0	0	0		
March		18	2	0	2	3	0	0(+1)		
April		6	0	0	0	0	0	0		
May		8	1	0	0	1	1	0		
June		6	0	0	0	1	0	0		
July		6	0	0	0	1	2	0		
Aug.		4	0	0	0	0	2	1	0	0
Sept.		3	0	0	0	0	0	1	0	0
Oct.		7	0	0	0	0	0	1	0	0
Nov.		19	1	1	0	1	0	0		
Dec.		26	0	0	0	0	0	0(+1)		
Jan.	1949	28	0	0	1	1	1	3	0	0
Feb.		27	2	0	0	1	0	0	0	0
March		77	22	3	2^{2}	5	1	20(+2)	9^{2}	2
April		23	2	0	1^{1}	1	2	6§	0	0
May		11	0	0	0	1	0	4	0	0
June		8	0	0	0	0	0	0		
July		8	0	2	0	0	0	0		
Aug.		13	0	0	0	0	0	0		
Total		383	33	8	17^{10}	16	9	38(+5)	9	3

*Superior numbers in this column indicate numbers of patients who also had elevated cold agglutinin titers.

†The number of autopsies in which attempts were made to isolate virus from the lung are shown in parentheses. An eightfold rise in titer of influenza A antihemagglutinins was demonstrated before death in one of the patients who died in March, 1949; serums were not available in the other four cases.

‡Superior numbers in these columns indicate the numbers of patients whose sera showed only a twofold rise in titers for the standard strain of the same type as that of the virus isolated from their throat washings.

§Two of these patients showed a fourfold rise in PR8 antihemagglutinins; serums of all other patients whose washings failed to yield a virus showed no significant titers or rises in titers with either PR8 or Lee.

or in the preceding or the following week. Three of the eight patients who showed a rise in titer against influenza B also became ill during the same period.

In addition to these results which were considered as very probably indicating infections with influenza virus of the corresponding type, there were twenty-five patients whose serums showed a significantly elevated titer (64 or higher) in the initial specimen obtained late in the disease and a twofold rise in titer or a twofold or greater drop in titer against only one of the strains in later specimens. Some, if not all of these patients may have had an influenzal infection with a virus of the corresponding type. In ten of these twenty-five patients the onset of clinical influenza occurred during, shortly before, or shortly after March, 1949.

There were seventeen more patients whose serums showed a fourfold or greater change in titer with both the PR8 and Lee strains. In ten of these seventeen patients, the serums also showed high titers of cold isohemagglutinins and the clinical picture was characteristic of primary atypical pneumonia. Only three of these cases occurred during the period of epidemic prevalence of proved influenza viral infections. Furthermore, there were among the 383 patients included in this study, eighty-two additional cases which were clinically characteristic of primary atypical pneumonia and in which the serums showed high titers of cold agglutinins but no significant changes in titer of antihemagglutinins for either the PR8 or the Lee strain of influenza virus.

The routine tests for agglutinin inhibition employing the PR8 strain of influenza A and the Lee strain of influenza B virus, therefore, revealed highly presumptive evidence of influenza viral infection in forty-one patients and possible infection with viruses of these types in twenty-five additional patients among the 383 patients included in this study. The results of these tests showed that there was a major concentration of cases of influenza A infections in Boston during March, 1949, that there were also some cases of infection with influenza B virus at that time, and that there were additional sporadic cases of infections with influenza A and influenza B viruses throughout much of the remaining period covered by this study. Nonspecific changes in titer of influenzal antihemagglutinins were noted in the serum of some cases of primary atypical pneumonia in association with the development of cold agglutinins, but such nonspecific changes in titer were observed in other patients whose serum did not have cold agglutinins and the great majority of patients with primary atypical pneumonia who did develop cold agglutinins failed to show significant changes in titer of influenzal antihemagglutinins.

Virus Isolations.—Attempts to isolate influenza viruses were made with throat washings obtained from thirty-eight patients and with suspensions of lung tissue obtained at autopsy from five additional patients. Strains of influenza virus were isolated from the throat washings of twelve of these patients but none were demonstrated in any of the lung suspensions. All but one of these twelve strains were isolated from washings obtained during March, 1949; nine of the strains isolated in that month were related to type A and two were related to type B. The remaining strain was isolated from a washing collected

in December, 1947; this was strain PW which was reported but not identified in a previous study[4] and has since been shown to be related to influenza B, as will be indicated later in this paper.

Acute and convalescent phase serums were available in all but two of the patients whose throat washings were used for virus isolations, and successive specimens of serum were obtained ante mortem from only one of those whose lung suspension was used for that purpose. Among the twelve patients whose throat washings yielded a strain of influenza virus, nine showed a fourfold or greater rise, while the remaining three showed only a twofold rise in titer of antibodies by the agglutinin inhibition test with the standard strain of virus of the homologous type. None of the latter three patients had elevated titers initially. The antemortem serums showed an eightfold increase in titer of antihemagglutinins for the PR8 strain, but only two of the twenty-six patients whose throat washings failed to yield a virus showed a fourfold or greater rise in antibodies against the PR8 or Lee viruses.*

The results of the attempts to isolate influenza viruses, therefore, confirm the serologic findings with respect to the prevalence and predominance of influenza A viral infections during March, 1949, and the occurrence of infections with influenza B virus during the same month. Only one sporadic case of influenza viral infection was demonstrated by the isolation of a virus during the two years of this study. These studies also confirm previous observations[1] that manifest cases of infection from which strains of influenza virus can be isolated may fail to give significant rises in antibodies detectable by the agglutinin inhibition test employing standard strains of influenza A and B.

Serologic Tests in Patients From Whom Strains of Influenza Virus Were Isolated.—More extensive serologic tests were carried out in the patients whose throat washings yielded influenza viruses. In addition to the routine tests for antihemagglutinins against the PR8 and Lee strains, complement fixation and *in ovo* neutralization tests were done in the serums of these patients with these two strains and, insofar as the supply of available serum permitted, all three tests were also carried out with the FM_1 strain, with the patient's own virus, and with one or two additional strains isolated from other patients during the same period. The results in the nine patients from whom strains of virus related to influenza A were isolated are shown in Table II.

None of the nine patients showed a significant rise in titer against the Lee strain of influenza B by any of the three tests employed. Only four of these nine patients (*Coop, Del, Arel,* and *Crow*) showed a significant rise in titer by all three tests against all of the A strains used. The only consistent findings in the serums of the remaining five patients were the significant rises in titer

*The throat washings from six of the patients were from institutional outbreaks and were originally sent to the laboratory of Dr. John F. Enders at the Children's Hospital in Boston. A strain of influenza A virus was isolated from the throat washings of one of these patients by Mr. Lawrence J. Kunz who also demonstrated a significant rise in titer of antihemagglutinins for PR8 in the serums of that patient (*Tren*) and of one of the patients whose washings failed to yield a virus. One of the strains of influenza B (*Cun*) was isolated by Mrs. Joan B. Daniels in the laboratory of Dr. F. Sargent Cheever at the Harvard Medical School. These seven cases all occurred in March, 1949, and the various findings were confirmed in this laboratory.

TABLE II. SEROLOGIC TESTS WITH SERUMS OF PATIENTS FROM WHOM VIRUSES RELATED TO INFLUENZA A WERE ISOLATED IN BOSTON DURING MARCH, 1948

SERUM		VIRUS ANTIGEN																	
		PATIENT'S OWN			COOP			DEL		TREN	FM_1			PR8			LEE		
PATIENT	DATE	HI	CF	EN	HI	CF	EN	HI	CF	EN	HI	CF	EN	HI	CF	EN	HI	CF	EN
Coop	Mar. 1	4	4	<4	4	4	<4	4	<4	4	32	<4	4	<4	<4	<4	<4	<4	<4
	Mar. 3	64	128	32	64	128	32	128	128	26	512	128	256*	32	128	8	<4	<4	<4
	Mar. 14	64	64	26	64	64	26	128	128	32	512	128	256	16	128	8	<4	<4	<4
McL	Mar. 5	<4	<4	<4	<4	<4	<4	—	<4	<4	64	<4	—	<4	8	—	<4	4	<4
	Mar. 11	32	32	41	32	32	32	—	64	32	256	64	256	4	32	<4	<4	8	—
	Mar. 21	64	64	100	64	32	64	—	32	32	512	64	162	4	32	<4	<4	8	<4
Weil	Mar. 7	8	<4	<4	4	4	<4	—	<4	<4	64	<4	<4	32	8	26	32	16	25
	Mar. 13	8	8	<4	4	8	<4	—	8	<4	64	8	<4	32	16	16	32	16	—
	Mar. 31	8	16	<4	4	16	<4	—	16	<4	64	16	4	32	32	32	32	16	16
Bass	Mar. 21	<4	<4	<4	<4	<4	<4	—	4	<4	16	8	8	16	16	160	<4	<4	<4
	Apr. 1	8	64	<4	8	64	<4	—	64	<4	128	64	32	128	128	408	<4	<4	<4
Del	Mar. 15	8	16	<4	8	16	<4	8	16	<4	51	32	<4	8	32	<4	8	16	—
	Mar. 23	32	512	26	64	512	40	32	512	—	4,096	512	1,024	128	512	4	16	—	<4
	Apr. 2	64	256	10	64	512	8	64	256	10	8,192	512	2,048	128	512	8	8	—	<4
Arel	Mar. 31	8	—	—	4	8	<4	—	8	—	32	8	—	<4	16	—	16	8	—
	Apr. 8	32	512	8	32	512	8	—	512	—	1,024	512*	64	32	512	128	16	8	—
Barb	Mar. 6	4	4	—	8	4	—	—	—	—	64	4	—	4	8	—	8	8	—
	Mar. 11	4	128	<4	8	64	<4	—	—	—	128	128	40	16	128	26	4	16	<8
	Mar. 18	4	128	<4	16	64	<4	—	—	—	128	128	16	32	128	32	8	16	<4
Crow	Mar. 19	<4	<4	<4	<4	<4	<4	—	<4	—	32	4	<4	<4	8	<4	4	4	—
	Apr. 28	16	64	16	64	64	32	—	128	—	512	128	645	16	128	16	4	4	—
Tren	Mar. 30	<4	<4	16	<4	<4	—	—	<4	16	16	<4	—	4	4	—	—	8	—
	Apr. 11	16	256	16	8	128	8	—	128	16	128	128	10	64	128	16	16	8	23

HI, Antihemagglutinin for chicken erythrocytes. CF, Complement fixation. EN, *In ovo* neutralization test.
—, Not done. *, Highest dilution positive (no end point).
Titers expressed as reciprocals of initial serum dilutions.
The inoculum in almost all of these tests contained between 100 and 333 ID_{50}.

of antibodies demonstrable by the complement fixation test and these were obtained with all of the type A strains. The hemagglutination and egg neutralization tests with these strains yielded irregular results.

Thus, the serums of *McL* failed to show a rise in antibodies against PR8 by either the agglutinin inhibition or the egg neutralization tests while such antibodies were demonstrated against the FM_1 and against the epidemic strains. Patient *Weil* failed to show a significant rise in titer by either of these tests against any of the A strains, including his own. Serums obtained from *Bass* showed a significant rise in titer by the hemagglutinin inhibition test with all of the A strains, but the egg neutralization tests yielded rises in titer only with the FM_1 and PR8 strains and not with the patient's own strain or with two other epidemic strains. The agglutinin inhibition tests done with the serums of *Barb* showed an eightfold rise in titer against PR8 and a twofold rise from an initial titer of 64 against FM_1 but no rise against the patient's own strain or against the strain isolated from *Coop*. The *in ovo* neutralization test likewise failed to demonstrate any antibody against the two latter strains, and the results of the neutralization test with the FM_1 and PR8 strains are uncertain in this case because acute phase serum was not available for comparison. The patient *Tren*, like *Bass*, showed a significant rise in titer by the agglutinin inhibition test with all of the A strains; in the *Tren* serums the egg neutralization tests, however, failed to demonstrate a rise in titer against the epidemic strains and showed no significantly elevated titers in the convalescent phase serum against either the FM_1 or the PR8 strain.

A sufficient supply of both acute and convalescent phase serums for extensive studies was available in only one of the two patients from whom a virus related to influenza B was isolated in March, 1949. The results in this case, *DeM*, are shown in Table III. Marked rises in titer of antibody were demonstrated against all of the B strains by all of the methods used, but no rises occurred against any of the A strains. Acute phase serum was not available in the case of *Cun*, the other patient from whom a type B influenza virus was isolated in March, 1949. However, elevated titers (mostly 32 or 64) were demonstrated in the convalescent phase serum of this patient by all three tests and against all of the B strains used but not against any of the A strains (titers of 8 or less). The results of many tests done with the serums of PW have already been reported.[4] They failed to show any changes in titer of antibodies against his own strain or against several type A and B strains by the agglutinin inhibition and complement fixation tests and by an *in ovo* neutralization test which utilized the yolk sac route of inoculation. Further tests carried out with these serums using the BON strain, the MB and MF strains of influenza B isolated in December, 1945, and the recently isolated *DeM* and *Cun* strains also yielded negative or indeterminate results.

In the serums of the patients from whom influenza viruses related to type A were isolated, therefore, the complement fixation test appeared to demonstrate antibody responses most consistently. However, while this test gave negative results with the influenza B strain in these cases, it failed to differentiate among the antigenically distinct strains of influenza A virus in these serums.

In previous studies made during an epidemic of influenza B, it was possible to demonstrate significant rises in titer to some type B strains in the serums of occasional patients by the complement fixation test when such rises could not be demonstrated by the agglutinin inhibition test with the same strains.[3]

TABLE III. RESULTS OF SEROLOGIC TESTS WITH SERUMS OF PATIENT DeM

VIRUS	TESTS	ACUTE PHASE	CONVALESCENT
DeM	HI	16	512
	CF	<4	1,024
	EN	<4	2,048
Cun	HI	16	512
	CF	<4	1,024
	EN	—	—
PW	HI	4	128
	CF	<4	1,024
	EN	—	—
Lee	HI	8	2,048
	CF	<4	1,024
	EN	8	2,580
BON	HI	4	128
	CF	<4	1,024
MB	HI	8	256
	CF	<4	2,048
MF	HI	<4	256
	CF	<4	2,048
PR8	HI	32	32
	CF	16	32
	EN	64	100
FM_1	EN	25	10
Tren	EN	<4	<4
Coop	EN	8	8

HI, Antihemagglutinin for chicken erythrocytes.
CF, Complement fixation.
EN, *In ovo* neutralization, using allantoic route of infection.
—, Not done.
Titers expressed as reciprocals of dilution end point.

Furthermore, during that outbreak, differences in responses to antigenically distinct strains of influenza B were noted in the serums of some of the school children but not in the adults.[3] Among the present cases, one of the three patients from whom influenza B viruses were obtained showed a marked rise in titer for all the B strains used, but not for any of the A strains by all of the three tests employed. In the single specimen of serum obtained in the second patient antibodies in apparently significant titers were also demonstrated against the B but not against the A strains. The third patient, PW, failed to show any significantly elevated titers or rises in titer of antibody in his serum against his own virus or against any of the A or B strains by any of the tests employed.

Antigenic Differences Among the Strains of Influenza Viruses.—The results of the serologic studies in the patients failed to reveal any distinct antigenic differences among the viruses beyond the general classification of the strains isolated in March, 1949, in relation to either type A or type B. Furthermore, strain PW, which was isolated in December, 1947, could not even be classified as to its type on the basis of these tests. It was, therefore, necessary to resort to serologic studies in animals immunized against the various strains. Rabbits were chosen for this purpose, in spite of their known shortcomings,

chiefly for convenience, previous studies having yielded highly satisfactory results when the egg neutralization test was used.[4] However, because of the much greater simplicity and economy of the agglutinin inhibition test and because it is now used almost exclusively in most laboratories, the results obtained with the latter test will also be presented for comparison. For this purpose the rabbit antiserums were used without preliminary treatment other than heating at 60° C. for twenty minutes.

Results of Agglutinin Inhibition Tests With Rabbit Antiserum.—The twelve recently isolated strains and the ten additional strains listed under Materials and Methods were used to prepare antiserums in rabbits. The antihemagglutinin titers in all of these serums against the recent strains are shown in Table IV and those obtained against the ten remaining strains are given in Table V. Because of variations in antihemagglutinin titers in normal rabbit serums when tested with different strains of influenza virus, the rabbits were bled immediately before immunization and the serum thus obtained from each rabbit was tested with the virus used to immunize that rabbit and also with several other viral strains. These results are also shown in Tables IV and V. Although marked rises in antihemagglutinin titer were not elicited in these rabbits, many suggestive differences and interrelationships were revealed among this array of strains. For convenience these will be indicated first with respect to the groups of antiserums and then with respect to the viral strains.

The antiserums prepared against each of the nine recently isolated type A strains all showed similar titers against all of these nine strains but no significant titers against the three recently isolated B strains. They all showed moderate titers against the Weiss virus, and high titers with the FM_1 strain and with strains EB and DW which were isolated in March, 1947, but little or no reaction with the PR8, Swine, Lee, BON virus or with the MB and MF strains which were isolated in December, 1945.

The anti-PW serum yielded high titers with all three of the recently isolated type B strains and also with the BON and MF viruses, but it gave only low titers with the Lee and MB strains and showed similar low titers with some of the A strains, viz. FM_1 and Weiss. This antiserum did not react with eight of the nine newly isolated A strains or with the PR8 or Swine viruses, but it showed what may be a significantly elevated titer with the *Barb* strain. The anti-*DeM* and anti-*Cun* serums showed elevated titers, with minor differences, against all of the B viruses and low titers with all of the A strains.

The antiserums prepared against the PR8, Weiss, Swine, FM_1 EB, and DW strains, in general, showed no significant titers against any of the type B viruses, including the recently isolated ones. They showed relatively low titers against almost all of the heterologous type A strains, except the anti-FM_1 serum which showed high titers with the *Tren* and *Barb* strains and the anti-Weiss serum which also showed a high titer with the *Barb* strain. It should be noted that the anti-Swine serum was of low titer and its reactions are difficult to interpret.

The anti-Lee and anti-MB serums reacted alike; they gave high titers with these two viruses and distinctly lower titers with the other B strains, including all of the recently isolated ones. The anti-BON and anti-MF serums were likewise similar but they gave higher titers with the newly isolated B strains than were obtained with the anti-Lee and anti-MB serums.

Turning now to the viruses, the nine recently isolated type A strains yielded similarly elevated titers with the antiserums against these strains but only low or insignificant titers against the remaining antiserums, including the anti-PR8 and anti-FM_1 serums. There were isolated exceptions: the *Barb* strains gave evelated titers not only with anti-Weiss and anti-FM_1 serum but also with anti-Lee and anti-PW serums and the *Tren* strain gave significantly elevated titers not only with FM but also with MF.

TABLE IV. TITERS OF CHICKEN CELL ANTIHEMAGGLUTININS OBTAINED WITH TWELVE RECENTLY ISOLATED STRAINS OF INFLUENZA VIRUS AND RABBIT ANTISERUMS PREPARED AGAINST THESE AND OTHER VIRUSES

RABBIT ANTISERUM	STRAIN OF VIRUS											
	COOP	MCL	WEIL	BASS	DEL	AREL	BARB	CROW	TREN	DEM	CUN	PW
Coop	*8/128*	8/64	8/128	64	128	64	64	64	8/128	8	16	<4/8
McL	64	*<4/64*	<4/64	32	16/64	64	16/64	64	8/128	16	16	4/4
Weil	64	32	*<4/64*	32	8/128	64	16/64	64	8/128	8	16	<4/4
Bass	64	32	64	*4/64*	8/64	8/64	64	8/64	8/64	4	4	--
Del	64	32	64	64	*8/128*	64	16/1,024	128	8/64	16	32	4/16
Arel	128	4/64	128	<4/64	4/128	*<4/128*	1,024	128	8/64	16	16	<4/4
Barb	8/64	64	8/64	8/64	8/128	128	*8/128*	8/128	64	<4/4	16	8
Crow	4/128	64	4/128	128	256	8/128	1,024	*4/256*	8/128	32	16	<4/4
Tren	8/128	128	4/64	128	8/128	4/128	128	128	*8/64*	8	16	4
PR8	16	8	16	8	16/16	16	64	16	16/4	8	4	4/<4
Weiss	16	<4/16	16	4/8	16	16	128	16	4/4	4/4	8	<4
Swine	16	16	16	8	16/8	8	16	16	16	4	32	8
FM_1	16	16	16	16	16	16	1,024	16	4/64	4	16	8
EB	32	8/16	32	8/16	32	8/32	32	8/32	32	4/<4	8	<4
DW	8/16	8/16	8/16	8	16	8/8	32	8/16	16	<4/4	16	8
Lee	16	8	16	8	16	16	128	16	16	8	<4/16	<4/4
BON	8	–	–	–	–	–	16	–	8	32	4/128	<4/16
MB	16	–	–	–	–	–	16	–	16	8	4/64	<4/4
MF	16	–	–	–	–	–	32	–	64	32	4/128	<4/8
DeM	8	16	16	16	16	16	32	32	16	*8/256*	16/128	32/128
Cun	8	4	8	8	8	16	16	16	16	256	*4/256*	128
PW	<4	<4	<4	<4	<4	<4	64	<4	<4	<4/64	64	*4/128*

Titers are given as reciprocals of initial dilution; when the control serum obtained before immunization was tested, its titer is shown as the numerator over the titer of the antiserum prepared in the same rabbit.

Titers in the serums of rabbits immunized with the homologous strain are shown in italics.

TABLE V. TITERS OF CHICKEN CELL ANTIHEMAGGLUTININS OBTAINED WITH CERTAIN WELL-KNOWN STRAINS OF INFLUENZA VIRUS AND WITH SOME STRAINS PREVIOUSLY ISOLATED IN THIS LABORATORY IN RABBIT ANTISERUMS PREPARED AGAINST THESE STRAINS AND AGAINST SOME RECENTLY ISOLATED STRAINS OF INFLUENZA VIRUS

RABBIT ANTI-SERUM	STRAIN OF VIRUS									
	PR8	WEISS	SWINE	FM_1	EB	DW	LEE	BON	MB	MF
Coop	8	32	8	256	256	128	8	4	4	<4
McL	8	32	<4/8	256	8/256	8/128	<4/8	4/8	<4/8	<4/8
Weil	8	32	<4/8	256	8/256	8/32	4/4	4/8	4/8	<4/4
Bass	<4/4	8/64	<4/<4	64/256	8/128	8/64	--	--	--	--
Del	<4/16	64	<4	32/512	512	16	16	8/16	4/8	<4/4
Arel	8	8/64	4	64/512	1,024	128	8	<4/8	4/8	<4/4
Barb	8	8/64	4	256	512	64	8	16	4	4
Crow	16	128	4	512	512	256	4/16	<4/4	<4/4	<4/<4
Tren	32	32	4	256	1,024	256	8	8	8	8
PR8	*<4/256*	128	<4/<4	16	32	64	8	16/16	8	4
Weiss	16	*4/256*	<4/<4	16	16	16	4	<4	<4	<4/<4
Swine	4	16	*<4/32*	32	32	32	8	16	<4/<4	<4/<4
FM_1	8	32	<4/4	*32/1,024*	512	16	4	16/8	4	<4
EB	4	8/8	<4	128	*32/512*	64	<4	<4	4/<4	<4
DW	8	32	<4	128	64	*8/128*	8	16	4/<4	<4
Lee	<4/<4	8	<4	8/16	16	16	*<4/128*	16	128	16
BON	8	16	4	8/16	16	16	16	*<4/64*	16	32
MB	4	8	<4	16/16	32	16	64	32	*<4/128*	16
MF	4	16	4	32/64	64	32	32	32	32	*4/128*
DeM	<4/8	32	4	64	32	32	16/64	32/256	16/256	32/256
Cun	<4/16	<4/16	<4/16	4/32	8/16	8/16	4/128	128	64	256
PW	<4	8	<4	16	<4	<4	16	8/128	16	128

Titers are given as reciprocal of initial serum dilution of antiserum; where the pre-immunization serum of the same rabbit was tested, its titer is shown as a numerator.

Titers in the serums of rabbits immunized against the homologous strain are shown in italics.

—, Not done.

The three recently isolated B strains, as a group, yielded the highest titers with the antiserums against these three viruses and no significant titers with any of the anti-A serums. They all gave better titers with anti-BON and anti-MF serums than with anti-Lee and anti-MB serums. The *Cun* virus yielded the highest titers with the anti-B serums but it also showed somewhat elevated titers with most of the anti-A serums.

The PR8 and Swine viruses gave significantly elevated titers only with the homologous antiserums but not with any of the other A or B antiserums, with the possible exception of the anti-*Cun* serum. The Weiss virus gave moderately elevated titers with the antiserums of all of the newly isolated A strains and particularly with anti-PR8 serum; it also gave slightly elevated titers of questionable significance with some of the B strains and with some of the other A strains. The FM_1 and EB strains reacted more or less alike; they gave high titers with the serums prepared against all of the newly isolated A strains and against the 1947 A strains but not with the antiserums against PR8, Weiss, Swine, or any of the B viruses. The DW strain reacted with most of the newly isolated A strains with anti-PR8 serum but not to any significant extent with any of the others.

The Lee and MB viruses acted alike and gave high titers with antiserums against these two strains and with anti-*DeM* and anti-*Cun* serums and lower titers against the BON, MF, and PW antiserums. The BON and MF strains also acted alike; they gave the highest titers with antiserums for these viruses and with those made against all three of the newly isolated B strains and somewhat lower titers with the anti-Lee and anti-MB serums.

On the whole, therefore, the results of the agglutinin inhibition tests with the rabbit antiserums were rather crude and indecisive. They did, however, suggest that there was a general antigenic uniformity among the nine recently

isolated type A strains and that they were all related to but definitely distinct from any of the earlier strains. Also, the *Barb* and *Tren* strains seemed to show some deviation from the others. The *DeM* and *Cun* viruses could readily be classed as type B strains; they appeared to be closely related to both Lee and BON but did not show complete antigenic similarity to either of them. The PW strain, though entirely distinct from the Lee virus, appeared to be related to the BON and MF strains but showed closer antigenic relationship with the newly isolated *DeM* and *Cun* viruses.

Results of in ovo Neutralization Tests With Rabbit Antiserums.—The same viruses and antiserums that were used in the previous tests, with a few exceptions, were also used in egg neutralization tests employing the allantoic route of inoculation. This test has the decided advantage that normal rabbit serums show no demonstrable neutralizing activity against any influenza viral infections. It is, therefore, much more suitable for the study of antigenic relationships, at least when rabbit antiserums are employed, and it was used successfully in similar studies previously in this laboratory.[3, 4] The results of all of the tests are shown in Table VI.

The antiserums prepared against all of the newly isolated type A strains reacted quite uniformly. They all neutralized each of these nine viruses and the FM_1 and EB strains in high and quite similar titers and neutralized the Weiss and DW viruses in low titers. However, they failed to neutralize the PR8 and Swine strains, the newly isolated B strains, and the Lee and BON viruses. Conversely, from the point of view of the viruses, the newly isolated A strains were all neutralized in equally high titers by the antiserums for each of these nine strains but they were all neutralized only in low titer by anti-FM_1 and anti-EB serum and not at all by any of the other anti-A serums (including the anti-Weiss and anti-DW serums) or by any of the anti-B serums.

The serums of the rabbits immunized with the three recently isolated type B strains failed to neutralize any of the type A viruses. The anti-*DeM* serum neutralized all of the B viruses in high titer; the anti-*Cun* serum neutralized the B strains in similar titer, except for the PW strain which it neutralized only in low titer, while the anti-PW serum neutralized all the B viruses except the Lee strain. Considering the viruses, on the other hand, the *DeM* and *Cun* strains were neutralized essentially to titer by the antiserums of all three of the recently isolated B strains, but they were neutralized only in low titer or not at all by the other B, or by any of the A strains. The PW virus was neutralized in high titer only by the homologous and the *DeM* strains, in low titer by the *Cun* virus, and not at all by the other B or any of the A strains.

These findings indicate, in a much more definitive manner than did the agglutinin inhibition tests, the antigenic identity of the 1949 Boston crop of influenza A strains. They show these strains to be closely related to the FM_1 and EB strains isolated in 1947 but quite readily distinguishable from these strains and quite distinct from all the other strains that were studied. These neutralization tests also indicate that the newly isolated *DeM* and *Cun* strains are quite similar to each other antigenically and although related to both Lee

TABLE VI. TITERS OF IN OVO NEUTRALIZATION OF INFLUENZA VIRUSES BY RABBIT ANTISERUMS USING THE ALLANTOIC ROUTE OF INFECTION

VIRUS		RABBIT ANTISERUMS																				
STRAIN	DOSE	COOP	MCL	WEIL	BASS	DEL	AREL	BARB	CROW	TREN	FM_1	PR8	WEISS	SWINE	EB	DW	LEE	BON	MB	MF	DEM	CUN	PW
Coop	333	*442*	442	256	610	358	1,630	4,096	2,048	2,048	11	<4	<4	<4	16	5	<4	<4	<4	<4	<4	<4	<4
McL	250	590	*1,460*	610	1,024	419	256	406	590	256	4	<4	<4	<4	9	<4	<4				<4	<4	<4
Weil	333	722	442	*128*	128	442	715	128	1,024	406	7	<4	<4	<4	32	<4	<4	<4	<4	<4	<4	<4	<4
Bass	333	431	431	152	*321*	431	512	128	406	1,024	6	<4	<4	<4	25	<4	<4				<4	<4	<4
Del	333	406	128	1,030	162	*162*	815	715	2,120	1,024	<4	<4	<4	<4	10	<4	<4				<4	<4	<4
Arel	790	512	162	256	512	406	*1,024*	512	2,048	646	32	<4	<4	<4	25	<4	<4	<4	<4	<4	<4	<4	<4
Barb	333	512	406	256	121	128	512	*512*	1,024	2,048	25	<4	<4	<4	32	<4	<4				<4	<4	<4
Crow	1,053	735	161	412	161	412	512	646	*2,048*	1,630	16	<4	<4	<4	32	<4	<4				<4	<4	<4
Tren	333	406	256	406	512	162	1,024	646	1,024	*512*	32	<4	<4	<4	16	<4	<4				<4	<4	<4
FM_1	1,053	4,096	512	512	646	128	2,584	512	6,500	2,048	*646*	<4	<4	<4	128	40	<4	<4	<4	<4	<4	<4	<4
PR8	105	<4	<4	<4	4	<4	<4	<4	<4	<4	<4	*256*	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4
Weiss	167	4	4	6	32	<4	11	4	64	11	<4	128	*512*	<4	<4	11	<4	<4	<4	<4	<4	<4	<4
Swine	3,333	4	<4	<4	<4	<4	<4	<4	<4	<4	<4	8	<4	*32*	<4	<4	<4	<4			<4	<4	<4
EB	500	512	1,024	646	646	256	1,024	406	256	647	147	<4	<4	<4	*512*	32	<4	<4	<4	<4	<4	<4	<4
DW	3,333	64	32	8	32	6	41	8	162	32	<4	<4	<4	<4	27	*162*	<4	<4	<4	<4	<4	<4	<4
Lee	50	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	*208*	<4	32	4	162	128	4
BON	7	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	8	*32*	8	32	>256	>256	>256
DeM	53	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	6	<4	8	16	*256*	162	64
Cun	637	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	4	8	512	*91*	40
PW	1,053	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	1,840	10	*128*

Dose of virus expressed as number of 50 per cent infective doses (ID_{50}) inoculated.

Titers of antiserums expressed as reciprocals of the 50 per cent end point; homologous titer in italics.

Normal serum obtained before immunization from each of the rabbits was tested with the virus used to immunize that rabbit and with one or more additional viruses; the titer was < 4 in every instance.

and BON and also to PW are quite distinct from all of them. The PW virus which was isolated in December, 1947, but not identified at that time was shown in these tests to be antigenically even more clearly distinct from any of the other strains; it appears to be more closely related to the *DeM* virus than to any of the other strains and also shows some relationship to the BON and *Cun* strains but it is clearly distinct antigenically from either of these strains and shows no antigenic relationship to the Lee strain in these tests.

DISCUSSION

The "routine" tests for antihemagglutinins as carried out in this study with the PR8 and Lee strains of influenza virus in the serums of patients with various acute respiratory infections apparently uncovered an appreciable number of sporadic cases of infections with influenza A and B viruses. The evidence is highly presumptive that such cases occurred in Boston during at least twelve of the twenty-four months covered by this study. No extensive or deliberate effort, however, was made to discover such cases or to determine the frequency of their occurrence.

The significance of changes in titer when they are found with both the A and B strains of influenza virus is not entirely clear from the present observations and obviously requires further study. However, in some cases, as when this occurs in association with high cold agglutinin titers in cases of primary atypical pneumonia, this phenomenon is probably nonspecific and it may represent influenza infection with cross reactions in other cases. This can be determined only by additional tests, which unfortunately were not done in the present study.

Attempts at isolation of virus likewise were not carried out regularly for the purpose of finding as many cases as possible; these were made only in a few patients strongly suspected of having influenza on clinical grounds and in some cases which were of special interest for other reasons. Only one sporadic case of influenza B infection was uncovered in this manner during the period of this study and, interestingly enough, this case would not have been identified as an influenza viral infection by the routine serological tests.

The inclusion of the FM_1 strain or a similar one in the routine agglutinin inhibition tests might have revealed some but probably not many additional cases as suggested by the findings in the case of *McL* (Table II), but in the great majority of cases the PR8 strain alone has given highly satisfactory results in influenza A infection in human cases as shown both in this study and in the 1947 cases.[4] These studies were done primarily in adults; it is possible that infants and children may give more specific antibody responses as suggested by some of the results obtained in an influenza B epidemic.[2, 3] In cases like that of *Weil* (Table II), neither the PR8 nor the FM_1 strain revealed any satisfactory antibody response by the agglutinin inhibition test, but the complement fixation test showed comparable rises in titer with both viruses.

Finally, it was possible by the routine Hirst tests to identify both influenza A and influenza B viral infections during March, 1949, and these findings were amply confirmed by isolation of viruses of both types from some cases. Some

cases of definite clinical influenza, verified by isolation of the virus, failed to show diagnostic changes in titer by these routine tests. The studies in the serums of the patients from whom viruses were isolated indicated that the complement fixation test though more elaborate and time consuming is a much more reliable test of influenza viral infection than the agglutinin inhibition test.

Preliminary studies made of cases of influenza in 1950 indicated that the majority of patients from whom viruses were isolated failed to show any rise in titer of antihemagglutinins with either the PR8 or Lee strains. Studies of these cases and of the strains isolated are in progress.

The studies on the antigenic relationships of the recently isolated strains indicated that serums from adult human patients with influenza viral infection are not satisfactory for distinguishing antigenic variants among either influenza A or B strains. Immune rabbit serums revealed some of these variations by the hemagglutination inhibition test, but in the present study this test by the simple method employed did not prove wholly satisfactory for that purpose. On the other hand, the egg neutralization test, utilizing the allantoic route of inoculation, proved highly satisfactory with the same rabbit serums and viruses. By this test it was possible to identify nine of the strains isolated in March, 1949, as type A strains which were quite uniform antigenically and related to but clearly distinct from the FM_1 strain and from both of the related variants which were isolated in this laboratory in March, 1947. The other two strains, *DeM* and *Cun*, isolated in March, 1949, were shown to be type B strains, but distinct from any other strains of that type that were studied. The PW strain, which was isolated in December, 1947, was identified as an entirely distinct variant of type B which was more closely related to the *DeM* virus than to any of the others that were tested. It was antigenically entirely distinct from the Lee virus in all of the tests that were applied.

It should be pointed out that other tests or serums prepared in other animals or different methods of treating the viruses or the serums may be equally satisfactory or may reveal even greater antigenic specificity, but the test as used here was reasonably simple and highly satisfactory. Many aspects of the problem of variations in influenza viruses have been reviewed recently by Briody.[7]

SUMMARY AND CONCLUSIONS

The experiences with the use of chicken cell agglutinin inhibition as a clinical laboratory test over a period of two years, using only the PR8 strain of influenza A and the Lee strain of influenza B virus, were reviewed. In a total of 383 patients, the results were considered "diagnostic" of influenza viral infection in forty-one patients, and evidence of possible influenza viral infection was obtained in twenty-five additional cases. The findings indicated that influenza was epidemic in Boston only during March, 1949, and that although influenza A viral infections predominated, some influenza B viral infections also occurred during that month. Sporadic cases of infection with either influenza A or influenza B also occurred in at least twelve of the twenty-four months of this study.

Virus isolations from clinical cases confirmed the prevalence and predominance of influenza A in March, 1949, and the additional occurrence of influenza B during that month. Only one sporadic case, in December, 1947, was discovered by isolation of a virus, and in that patient the serologic results were not diagnostic.

A more detailed serologic study in the patients from whom influenza viruses were isolated indicated that the complement fixation test is more reliable than the agglutinin inhibition or egg neutralization test for the serologic diagnosis of influenza. However, this test does not reveal antigenic differences among strains, at least in adult patients.

The antigenic relationships of twelve newly isolated viruses were studied with the use of immune rabbit serums. Agglutinin inhibition tests with heated but otherwise untreated serums gave suggestive antigenic patterns, but the results were not satisfactory. The *in ovo* neutralization test, utilizing the allantoic route, proved highly satisfactory with the same immune rabbit serums. The latter tests showed that nine of eleven strains isolated in March, 1949, were antigenically identical; they were related to the FM_1 and EB strains of influenza A isolated in 1947 but were clearly distinct from these and from all other type A strains that were tested. The other two strains isolated at that time were antigenically closely related to each other and, although related to both the Lee and BON strains of influenza B, were clearly distinct from either of them.

The PW strain, isolated in December, 1947, was shown to be antigenically distinct from any of the other strains; it was, however, related to the two type B strains isolated in March, 1949, and, to a less extent, to the BON strain.

REFERENCES

1. Finland, M., Barnes, M. W., and Samper, B. A.: Influenza Virus Isolations and Serological Studies Made in Boston During the Winter of 1943-1944, J. Clin. Investigation **24**: 192, 1945.
2. Finland, M., Barnes, M. W., Meads, M., and Ory, E. M.: Serologic Studies of Influenza Made in Boston During the Winter of 1945-46, J. LAB. & CLIN. MED. **33**: 14, 1948.
3. Barnes, M. W., Morgan, H. R., and Finland, M.: Isolation and Identification of Influenza Viruses During the Epidemic of December 1945, J. LAB. & CLIN. MED. **33**: 309, 1948.
4. Morgan, H. R., Barnes, M. W., and Finland, M.: Antigenic Differences Among Influenza A Viruses, Including Serologic Response of Patients, J. LAB. & CLIN. MED. **33**: 1212, 1948.
5. Hirst, G. K.: In Vivo Titrations of Influenza Virus and of Neutralizing Antibodies in Chick Embryos, J. Immunol. **45**: 285, 1942.
6. Reed, L. J., and Muench, H.: A Simplified Method of Estimating Fifty Percent Endpoints, Am. J. Hyg. **27**: 493, 1938.
7. Briody, B. A.: Variations in Influenza Viruses, Bact. Rev. **14**: 65, 1950.

OBSERVATIONS ON THE EPIDEMIOLOGY OF INFECTIOUS HEPATITIS

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INFECTIOUS hepatitis is a disease of major importance in the United States. An awareness of its significance developed rapidly during and subsequent to World War II. The frequency and widespread distribution of infectious hepatitis have stimulated intensive investigations of many aspects of this disease, especially those which have a bearing on the mode of dissemination. Throughout the war period one of us (E. M. C.), under the supervision of the Division of Preventive Medicine of the United States Army, made observations pertaining to the epidemiology of hepatitis among United States personnel in the European Theater of Operations. The interpretation of these data, and that of others obtained in wartime, is complicated by the varying situations created by military operations. Other studies have been made in local areas of the United States where the population was static. Different observers have interpreted the information available to favor either the airborne or the gastrointestinal route of transmission. However, the mechanism of dissemination in nature is not known with certainty. More observations in the field are important for the ultimate solution of this problem. Several review articles, which adequately summarize the important contributions to the subject, are available.[1-5]

Valuable information about the nature, pathogenesis, and cause of infectious hepatitis has been accumulated in recent years. There is reason to believe that the causative agent is of virus origin. It has been demonstrated in the blood and stools of patients. The infection can be transmitted to human beings by the oral and parenteral routes.[6] Information of crucial importance for the solution of its epidemiology will result from the studies which await successful isolation and identification of the specific etiologic agent.

This report is concerned with certain observations which were made on the occurrence of infectious hepatitis in two widely separated areas in Wisconsin. The data can be interpreted to favor either the airborne or the gastrointestinal routes of dissemination. The evidence does not seem to provide conclusive support for either theory.

METHODS

The study was carried on during the summers of 1948 and 1949. Contact with developments in the areas concerned is being maintained. For the purpose of this report, the observation period terminated in August, 1949. The survey was begun in July, 1948, in a north central section of the state. In the course of events, the greater part of Lincoln County

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was included. Later in the summer of 1948 the study was transferred to a region in the southwestern part of the state. There it was found that cases of infectious hepatitis had been localized to a small area of Wiota Township, Lafayette County. (Fig. 1.) The two regions are 221 miles apart and present obvious differences in social, environmental, and geographic aspects. The populations of both localities had remained static for a number of years.

Fig. 1.—Outline map of Wisconsin. The blocks indicate the north central and southwestern areas studied.

The region in the north central section extended to involve an area and a population which was larger than that originally intended for study. These circumstances complicated the problem and added to the difficulties of interpretation. The other (southwestern) section was limited to a small area of about 4 square miles and consequently presented a less complicated field for study.

The first step in the survey of each region consisted of consultation with the local physicians. Through them a few patients who had had infectious hepatitis during the weeks preceding were discovered. The judgment of the physicians was essential to establish the presence of infectious hepatitis. This disease was made reportable to the State Board of Health after the period involved. During this time the schools did not keep individual health records. Consequently, most of the information was obtained by interview with individuals who were thought to have had the infection and with members of their families and other

TABLE I. TIME SEQUENCE AND LOCATION OF INFECTIOUS HEPATITIS, NORTH CENTRAL AREA

YEAR AND MONTH	CITY			RURAL DISTRICTS†												TOTAL
	SW	WEST	EAST	S.S.	I.	C.V.	C.	H.S.	F.	P.R.	G.	G.F.	C.F.	I.T.	F.R.	
1947																
Jan.		1														1
Feb.		2														2
Mar.		1														1
Apr.	1	2		1	1											5
May			1													1
June		1	1													2
July		1	2													3
Aug.		3	1													4
Sept.		1	1			1										3
Oct.	1	1	1													3
Nov.		1														1
Dec.		2	5													7
1948																
Jan.	1	2	7				1	1								12
Feb.		1	9			1			1							12
Mar.	1	1	9	1		2				6						20
Apr.	1	1	1			3				7						13
May			4*			1			2	6	1					14
June			4							2		4	1			11
July		1										1				2
Aug.																0
Sept.			1*											1		2
Oct.		1												3		4
Nov.														3		3
Dec.														10		10
1949																
Jan.	1*													7		8
Feb.														4	1*	5
Mar.														1		1
Apr.			1								1*			1		3
May			1											5		6
June		1*												4		5
July			2*								1					3
Aug.	1															1
Total	7	24	51	2	1	8	1	1	3	21	3	5	1	39	1	168

*One case of each considered due to serum transmission; total: 7.

†The letters represent separate districts.

contacts. The leads, suggested by these interviews, resulted ultimately in the discovery of most, if not all, of the persons in the region who had had hepatitis with jaundice. The diagnosis of infectious hepatitis is admittedly presumptive since it depended upon history and other circumstances. When subjects were seen after recovery, the history of jaundice during the illness was considered mandatory for inclusion in the series. The individuals who may have suffered subicteric hepatitis could not be included. Two small children, in whom other circumstances seemed to justify the diagnosis of nonicteric hepatitis, were the only exceptions.

A questionnaire was used as a guide for each interview. Data were sought relative to the nature of illness, sequence of cases, contacts, movement of the individual prior to and after illness, individual and family habits, source of food, milk, water, and other environmental influences. In addition to that made by the observers, a sanitary survey of each area was made by the District Sanitary Engineer. Water was obtained for bacteriologic analysis from wells, mains, public places located in or near the home where cases of the disease occurred, and from other homes in near-by areas. Information was obtained relative to the geographic and geologic features of the areas, nature and condition of wells and other water supplies, occurrence of communicable diseases, character of industrial and agricultural practices, diseases of livestock, and weather reports. A brief survey of the records of water analyses from all parts of Wisconsin was included.

For control, and to reduce the possibility that cases had been overlooked in the city of Merrill, visits at random were made to homes in the city where infectious hepatitis was not suspected. To this end, sixty families were visited without conscious selection. This effort did not uncover other cases.

RESULTS

Occurrence of Infectious Hepatitis in the North Central Section.—The physicians in the city stated that cases of infectious hepatitis had occurred sporadically in the community for several years. However, a significant increase in incidence began in the fall of 1947.

The methods employed led to the discovery of 168 individuals who had had infectious hepatitis, as determined by the criteria indicated, during the period from January, 1947, through August, 1949. The geographic distribution of these within the area and the time sequence of their occurrence are summarized in Table I. The region was divided into two parts: the urban (Merrill) and the surrounding rural localities. The rural communities, in which the disease occurred, consisted of several districts. The general features of this distribution are presented in Figs. 2 and 3.

In the city, eighty-two individuals were discovered who are believed to have had the disease during the period indicated. Sixty families were represented, in which there were a total of 292 members. This represents an incidence of infection within families of 28 per cent.

In the surrounding rural communities, eighty-six members of forty farm families had had the disease. A total of 212 persons were represented in the families, an incidence of infection of 41 per cent. In one family seven members developed the disease. For the area as a whole the disease incidence was 33 per cent in the families involved. (Table II.) Cases of infectious hepatitis in the rural areas appeared a little later, in general, than those in the city. (Table I.) The observations suggest that dissemination may have proceeded from the city into the surrounding rural communities. In Merrill, cases appeared most frequently in the winter months. The highest incidence occurred

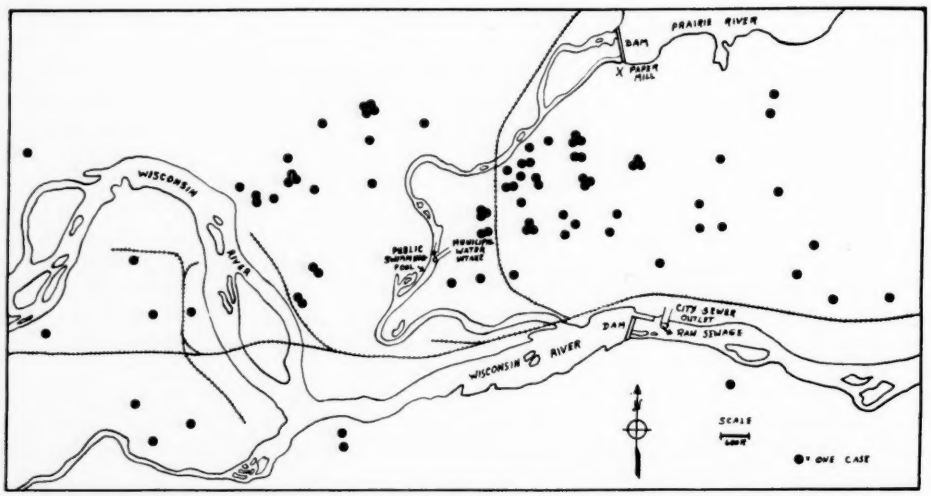

Fig. 2.—Sketch of the city of Merrill in the north central section, indicating the general geographic configuration and the location of cases of infectious hepatitis. Eighty-two cases in sixty families were discovered.

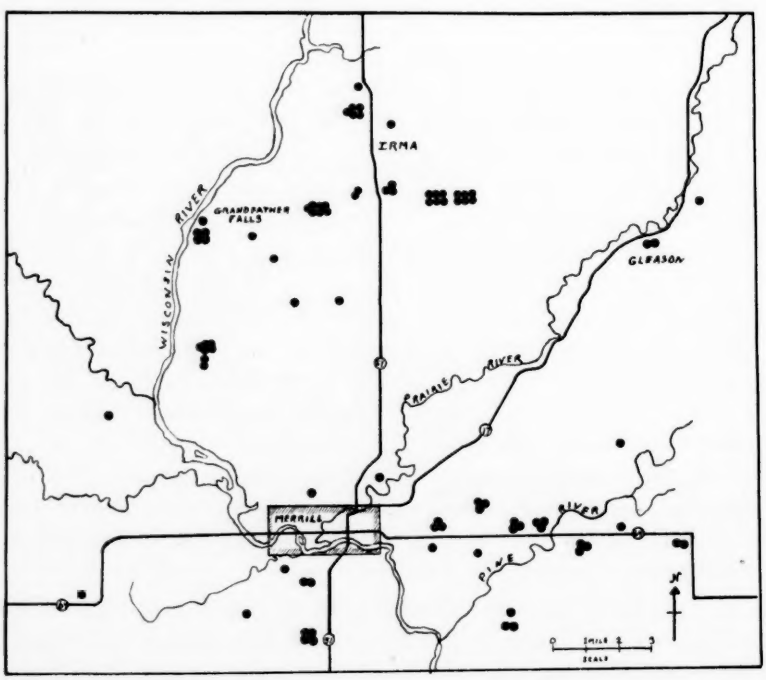

Fig. 3.—Sketch of the north central area indicating the relationship of rural localities to the city and the distribution of cases of infectious hepatitis in the rural areas.

in March, 1948. In the rural areas it was most frequent during March, April, May, June, and December, 1948, and January, 1949.

The greatest number of cases occurred among subjects between 10 and 30 years of age. Children under 5 and adults over 50 rarely developed the recognizable syndrome. (Table II.) Only two of the fifty-five children under 5

TABLE II. AGE AND ATTACK RATE OF INFECTIOUS HEPATITIS IN FAMILIES,* NORTH CENTRAL AREA

AGE	CASES		FAMILY CONTACT	
	NUMBER	PER CENT	NUMBER OF INDIVIDUALS	PER CENT WITH DISEASE
0 – 4	4	2	55	7
5 – 9	32	19	86	37
10 – 14	43	26	74	58
15 – 19	27	16	60	45
20 – 29	21	12	41	51
30 – 49	33	20	139	24
50 and over	8	5	49	16
Total	168	100	504	33

*All cases occurred in 100 families.

years in affected families became jaundiced. Two other children were discovered, aged 1 and 2 years, who were believed to have had infectious hepatitis without icterus.

Time Sequence of Occurrence in Rural Families: The rural areas in which cases were found were arbitrarily divided into twelve districts. Most of the residents of these districts had had opportunity for contact with those of other rural districts and of the city. The circumstances surrounding the occurrence of the disease in three of these rural districts were as follows.

(1) In a district south of the city, designated C.V. in Table I, eight individuals developed the disease during the period of September, 1947, to May, 1948. The grade school located there had an enrollment of twenty-six pupils. During the school year, 1948-49, four of them had infectious hepatitis. A review of the developments in this district revealed the following.

A school child from the city visited a home in C.V. at the time of onset of his disease (Jan. 16, 1948). This child had visited the same home in C.V. on Jan. 1, 1948. On February 20 a child of this rural family who had slept with the visitor developed anorexia, vomiting, fever, and general malaise, but no jaundice. About March 21 his father became ill with anorexia, headache, and fever, followed by jaundice. The total duration of his illness was short. The mother developed similar symptoms about April 6. By April 16 she had marked jaundice, fever of 104° F., and dark urine, with prostration and fatigue. The jaundice persisted for several months and she stated, four months later, that she still felt worn out and ill. The fourth member of this family was a daughter, aged 8, who had mild symptoms and slight jaundice about April 15. Recovery was prompt. Three children in the family, ages 7, 5, and 2 years, did not develop symptoms. All of the members of this family in C.V. had visited the city but did not know of contact with the disease there. The children attended the grade school in the rural district.

The disease appeared without other known contact in two other families of the same region (C.V.) whose children attended the grade school. All of them had also visited the city. In the first of these families, a boy, aged 7, became ill with jaundice on March 15. His sister, aged 15, who was not in school because of St. Vitus's dance, contracted the disease about the seventh day of May. Although not attending school, her associates were not limited to the

family. She went to the city to shop and to movies, as did the other children in the community. Six members of this family remained well. In the middle of May one child of a third family developed evidence of hepatitis.

The only case of hepatitis known to have occurred in this region prior to the onset of the group of eight described occurred in a single individual, aged 17 years. He had jaundice in September, 1947, while he was working in the foundry in the city. There was one other case among the foundry's employees on about the same date. His family denied contact with other cases of hepatitis.

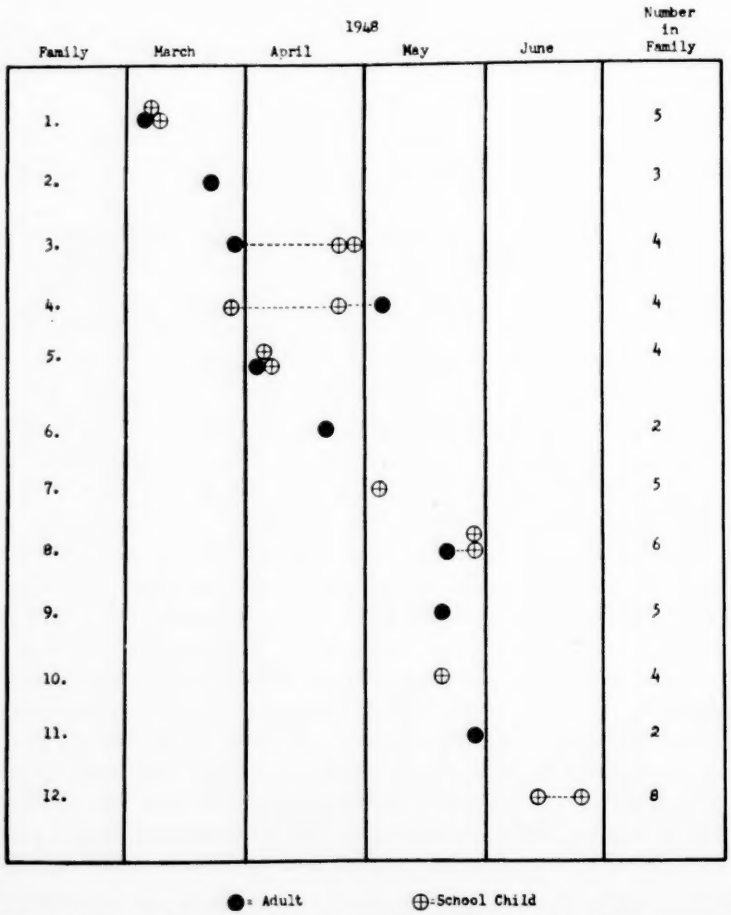

Fig. 4.—Time sequence and summary of occurrence of infectious hepatitis among families in a rural area (P.R.) in the north central section.

(2) The Pine River Township is located to the east of the city. (Fig. 3.) A group of fifteen families were considered as a unit because of social and blood relationships, although four of them lived in the city. Frequent group parties were held in rotation. There are fifty-five individuals represented in the group. Of these, sixteen, including two young children without jaundice, developed the disease between March 1 and June 1, 1948, an attack rate of 29 per cent among

the fifty-five members of the fifteen families. Eight of these families were involved, an attack rate of 51 per cent. A cousin, aged 11, who lived in another district, but who had attended a social gathering in March, developed the disease early in May. The members of his family and others in this district were not affected.

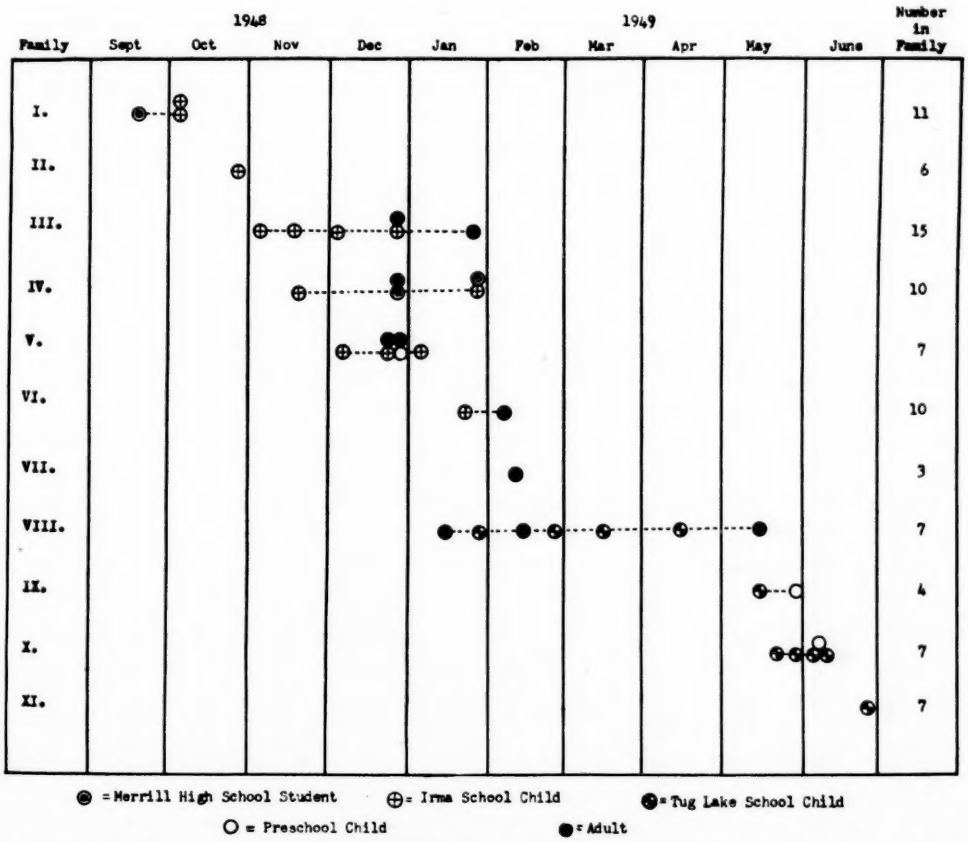

Fig. 5.—Occurrence of infectious hepatitis in the families of the I.T. rural locality in the north central area.

The disease also appeared in three families in the locality which were not included in the "social group." The contact appeared to have been limited to the schools. Five children and one mother were involved. The occurrence of the disease and the time sequence between the onset among members of the twelve families described are summarized in Fig. 4.

(3) An area known as the Irma and Tug Lake School Districts (designated I. T. in Table I), twelve miles to the north of the city, experienced a sustained outbreak of infectious hepatitis beginning in September, 1948. (Fig. 3.) Between that time and June, 1949, thirty-nine cases occurred among the members of eleven families. The time sequence of onset and the incidence of the disease in the families involved are presented in Fig. 5. In each instance the first case

to appear in a family was a child attending the grade school. Two members of Family VIII, for which a contact source of infection was not evident, spent the Christmas holiday with Family V. In most families cases occurred at intervals of two to four weeks. Occasionally the onset of two or more cases occurred at the same time after an original case. The first case in this outbreak was a child who attended the high school in Merrill. On the first Friday in July she was present at an all-day meeting of the church in a near-by district. She was also present at an all-day meeting of a church in the city on the first Friday of September, 1948. On the Tuesday after Labor Day she returned to high school. On the 17th of September, 1948, she became ill with a disease resembling infectious hepatitis. On October 4 her brother became ill, and a few days later a sister developed similar symptoms. These siblings attended the grade school in Irma. Subsequently the members of ten other families in the district contracted the disease.

Prior to September, 1948, sporadic cases had occurred in five individuals who lived within the region to the north of the city. In these the illness had occurred between the spring of 1947 and July, 1948. They are included in Table I in separate columns to indicate separate districts because a connection between them and other cases was not recognized.

Occurrence of Infectious Hepatitis in the Southwestern Section.—The survey of this region revealed that the disease had been localized to an area of approximately 4 square miles. (Figs. 1 and 6.) Cases had appeared over a period of fourteen months, Dec. 2, 1945, to Jan. 25, 1947. (Fig. 7.) The disease has not been recognized in this area subsequently. The search failed to discover evidence of its presence elsewhere in the township, or within a radius of several miles. In the region, thirty-one individuals were discovered who are believed to have had infectious hepatitis. These represented ten of the forty-nine homes in the district, and four near by (Argyle Village, Argyle Township, east of Woodford, and South Wayne). (Figs. 6 and 7.) All of the fourteen families are directly involved with the small area noted. There were seventy-four persons in the affected homes, an attack rate of 42 per cent. (Table III.)

The age distribution was essentially the same as that described for the north central area. The greatest number of cases occurred during the winter and spring months. In March, 1946, the disease appeared for the first time

TABLE III. AGE AND ATTACK RATE OF INFECTIOUS HEPATITIS IN FAMILIES, SOUTHWESTERN AREA

AGE	CASES		FAMILY CONTACT	
	NUMBER	PER CENT	NUMBER OF INDIVIDUALS	PER CENT WITH DISEASE
0 – 4	1	3	12	8
5 – 9	5	16	15	33
10 – 14	1	3	5	20
15 – 19	5	16	6	83
20 – 29	8	26	10	80
30 – 49	11	36	17	65
50 and over	0	0	9	0
Total	31	100	74	42

in four families. The interval between the onset of the disease in individuals within families and throughout the area is indicated in Fig. 7. In families, cases tended to appear at an interval of two to four weeks. The last family noted in Fig. 7 is an exception. More than three months separated the last case from that preceding. A grouping of "secondary" cases was rare. In one instance the onset occurred fourteen days postpartum. There is no evidence that the infant contracted the infection.

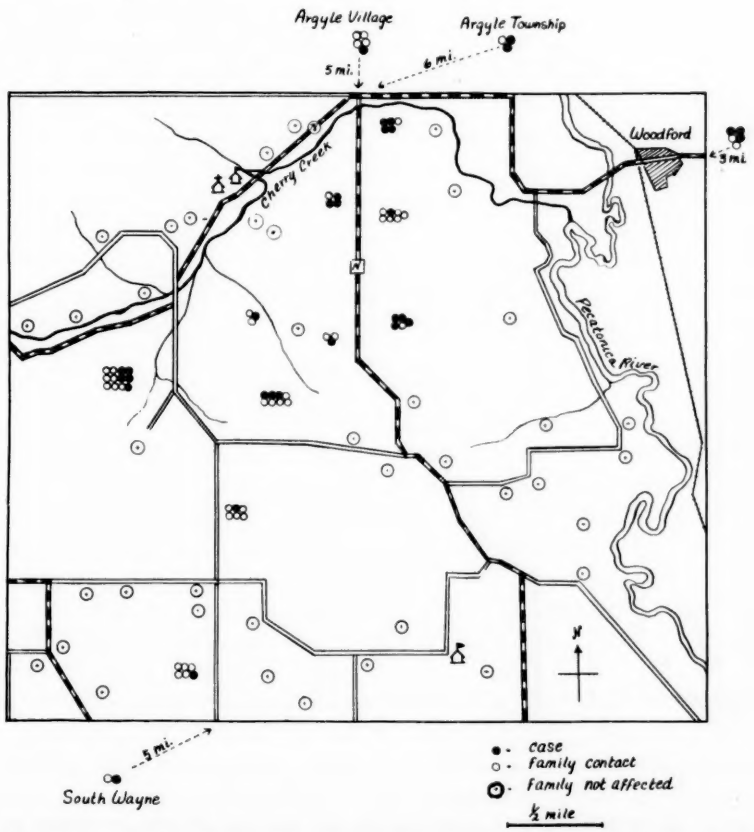

Fig. 6.—Map of the small southwestern region, indicating the general geographic features, the location of the farms in the area, and the distribution of cases of hepatitis.

The limited geographic distribution of the disease in this area is striking. With the exceptions to be noted, cases were limited to those who lived between the Pecatonica River and Cherry Creek and were most frequent along County Route N. Along this road only two families remained unaffected. Almost all of the forty-nine families in the area attend the same church. Infectious hepatitis, in recognizable form, was limited to those south of Cherry Creek. There was a tendency to involve several members of a family. All of the members of the other thirty-five families escaped infection, as far as could be determined. Environmental circumstances and the opportunity for contact were apparently almost identical.

Infectious hepatitis developed in the members of four families who lived outside the 4 square mile area. (Fig. 6.) All are closely related to one or more of those in the area. Members of these families had lived in a home there when cases of jaundice were present. The case, noted in a family in Argyle Village to the north, was that of a man who had been the hired man on the farm of Family III and lived with the family when the disease was present. He left the farm on the 1st of May, 1946, and went to live with his sister's family

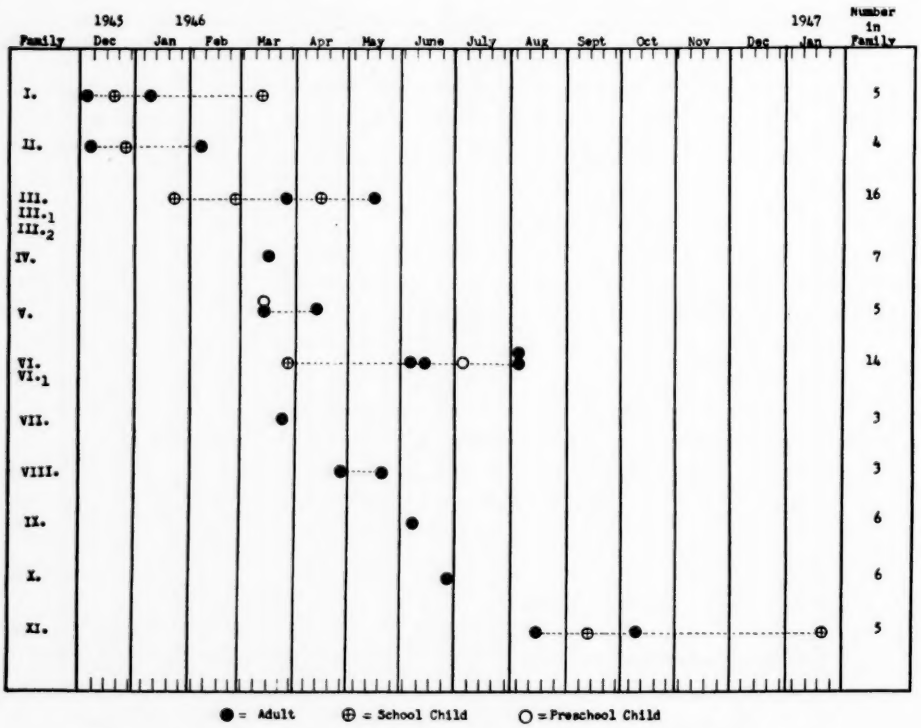

Fig. 7.—Occurrence of hepatitis in the families in the southwestern locality.

in Argyle. In about three weeks he became jaundiced; no members of his sister's family or other contacts contracted the disease. A brother and sister of the family to the east of Woodford were adult members of two affected families within the 4 square mile area. The disease did not extend to neighboring homes and there were no cases in the village of Woodford. The patient in South Wayne developed her disease on Aug. 1, 1946, while teaching in the parochial school in Wiota village. She was living in the farm home of Family VI at the time. Six of the twelve members of Family VI were ill during the outbreak. No other cases of hepatitis were discovered in South Wayne. The first case in the family in Argyle Township was the teacher in the grade school. (North of the church, Fig. 6.) She became ill with hepatitis during the last of April, 1946. A month later her brother had similar symptoms. Spread of the infection beyond this family in Argyle Township was not discovered.

The disease was limited to single adult members in some of the families. (Fig. 7.) Family II represents the parents of Family IV. The first case in Family VIII was a teacher in the local school. Families IX and X apparently did not have close contact with other affected families in the area. These two farms were located farther to the south. The father in each family was the only one affected. One raised beef cattle instead of dairy cattle. This farmer (IX) frequently visited with his neighbors at the cheese factory near the church on Cherry Creek. The other (X) had little contact with his neighboring farmers but often visited Family IX. The school children of these homes attended high schools outside of the area. Cases of infectious hepatitis were not discovered in these schools. The hired man in household X was a relative and had frequent contact with two other families whose members were affected.

Direct Contact Between Cases.—

North Central Section: With few exceptions, patients gave a history of contact with others who had been in an early stage or just prior to onset of the disease. Contact took place under various circumstances: at work, at school, at social functions, and between playmates or members of the family. In the city the greatest number of children involved, twelve and thirteen respectively, attended two schools. Both of these were located on the east side. Cases appeared among the students of five other schools in the city as well: seven, six, one, one, and two cases in each school, respectively. In all instances the numbers involved were very few in comparison with the enrollment. The disease was fairly evenly divided among adults and children. The nature of the contact between cases in three of the rural districts has been briefly described. Sometimes individuals, children and adults, did not develop the recognizable disease even after sleeping with patients during the acute stage of their illness.

The frequency of occurrence within families is an interesting feature. The observations suggest that the disease is related to the home more than to occupation, school attended, or other factors.

Southwestern Section: It was demonstrated that almost all of the thirty-one patients with infectious hepatitis had had prior direct contact with another similar patient during the latter's disease. With the exception of two families, all were members of the same church. The elementary school children attended the local school. Six of the twenty pupils and the teacher developed the disease. In only two of the fourteen families was a school child the primary case in the respective home. Close contact between neighboring homes was frequent. The tendency for the disease to present a pattern of occurrence by families was even more striking in this small rural locality than that observed in the north central region. The thirty-one cases were traced to ten families in the area of 4 square miles. Seventeen of these individuals were related and most were siblings, or the offspring of siblings. The spouse was sometimes involved as well. More intimate contact would be expected between members of related families than with others. This is probably not a major factor since contacts between neighboring farm homes seemed to be nearly as close. The absence of dissemination of infection to surrounding areas is noteworthy. Cases of infectious hepatitis were not recognized in individuals living north of Cherry Creek. (Fig. 6.)

Environmental Features.—

Description of the North Central Section: Lincoln County, located in the north central part of the state has an area of 885 square miles. In most parts the surface is a gently undulating plain. The northern half of the county is gently sloping. The southern and southeastern part is covered with glacial drift and the associated alluvial sands and gravels. In the southeastern part of the county the surface is characterized by prominent valleys. The drift overlying the granite is relatively thin in many places. Rock rapids occur at Merrill and Grandfather Falls and at many places along the tributaries of the Wisconsin River. The surface of drift and alluvial sand and gravel is the principal water-bearing formation. It furnishes an abundant supply at the relatively shallow depth of 20 to 50 feet.[7]

The city of Merrill has a population of approximately 10,000. It is located upon the gravelly and sandy terraces of the river formation. The Prairie River divides the city into east and west sections and empties into the Wisconsin River within the city. (Fig. 2.) A tabulation of the registered voters indicates that 56 per cent live on the east and 44 per cent on the west side of the Prairie River. Within the city, fifty-one cases of infectious hepatitis occurred on the east side and thirty-one on the west. In terms of population this suggests a predominance on the east side, but it is not statistically significant. To the west of the river the elevation is generally about 20 feet higher than to the east. As shown on the map, the east side of the city is surrounded on most of three sides by the Prairie and Wisconsin rivers. Underground seepage between the two rivers is a theoretical source of well water contamination. When the rivers flooded several years ago, a portion of this low eastern section was underwater.

At Irma, the wells are from 10 to 40 feet deep. In districts to the north they are from 20 to 40 feet in sand and gravel, and those to the east are generally from 20 to 40 feet in the drift.

The homes in the city of Merrill are typical of those of average, small, midwestern cities. The surrounding communities are characterized by relatively prosperous farms. The Irma-Tug Lake area, twelve miles to the north, is essentially rural.

Description of the Southwestern Region: Lafayette County has a different geologic configuration.[7] The surface is a deeply trenched upland plain, rising to a general elevation of 1,100 feet above sea level. The Pecatonica River has a flat-bottomed valley, generally less than half a mile wide. The main valleys are usually narrow. The altitude of the valley bottom ranges from approximately 780 feet to 880 feet. The surface deposit ranges between 0 and 10 feet in thickness. In the valley bottoms the river deposits of sand and gravel may attain a thickness of 200 to 300 feet. The principal water-bearing strata are the Platteville-Galena limestone and the St. Peter's limestone formations. Some wells obtain their supply from the Lower Magnesium. Springs are common where the shale strata outcrop in the valley. It is a very prosperous farming community and the dwellings, barns, livestock, and implements are well attended.

Water Supply, North Central Section: During the period under consideration the municipal water supply was obtained from the Prairie River, upstream from the city. (Fig. 2.) Untreated river water was invariably "unsafe" by *Escherichia coli* count standards. The local swimming hole is located across and slightly downstream from the water supply intake. The river water is high in organic content and required several procedures, including chlorination, for purification. After treatment this water was safe, but sometimes was slightly colored and had an unpleasant taste. Many families preferred to use water from privately owned wells. The municipal water mains did not extend throughout the city so that some sections were without the municipal supply. All of the homes in rural areas provided their own sources of water.

Privately owned wells are distributed widely throughout the city. These were used by both homes and places of business. Registration of private wells was not required and the number was unknown. The wells are dug, driven, or drilled. Most were between 20 and 30 feet in depth. Few privately owned wells had been inspected or water from them subjected to analysis for potability.

Outdoor privies are a common feature in the urban as well as the rural homes. Often a privy and a well are located close together. Eleven units of this type were found in the homes surveyed where infectious hepatitis had occurred. In one instance, two privies were within a 20 foot radius of a shallow well. Private wells were found to be the usual source of water for thirty of the sixty families in the city where hepatitis occurred. A sanitary survey of the conditions involving the source of water for twenty-five homes was made by the District Sanitary Engineer on Aug. 23-25, 1948. The following are examples of opportunities for gross contamination which he found: sewage backed up into the basement where a well was located; water from the near-by Prairie River backing up into wells; a privy located 10 feet from a well; a privy on an elevation 10 feet above and 50 feet away from a well; and old wells being used as drainage pits in close proximity to the new supply. Only one well was found sealed effectively. A review of the distribution of wells which were considered "unsafe" suggests that all of those within 100 feet of the river should be suspected.

During the period of study in 1948, analyses of drinking water obtained from private wells and other sources in the area were made. (Table IV.) These represent the results obtained on single specimens of water, and therefore, cannot accurately reflect the conditions present. In addition to the results noted in Table IV, water from twenty-three wells, which were used by the public, was examined. These included wells in parks, factories, taverns, and the County Fair Grounds. Six of the twenty-three specimens were reported "unsafe." Water from forty-four wells located in the rural area surrounding the city was submitted for analysis. Sixteen were reported "unsafe." Included in these were wells on twenty-five farms where no cases of hepatitis had occurred; ten of these were reported "unsafe." Two dairies had wells. The water from both was reported "unsafe." The water obtained from a well on the premises of a milk plant was reported "unsafe." Well water from one of five taverns was reported "unsafe"; that from one of nine rural schools was reported "unsafe," and that from one of two public service establishments in the rural area was reported "unsafe."

TABLE IV. ANALYSIS OF WATER OBTAINED FROM PRIVATE WELLS IN THE CITY

Per cent of families using private wells (estimated)	44
Number of families in which hepatitis occurred	60
Number of these who used private wells	27
Number of wells tested for potability	14
Number from which water was reported unsafe	5
Per cent reported unsafe	36
Private wells used by families without hepatitis; number tested	76
Number from which water was reported unsafe	10
Per cent reported unsafe	13
Private wells throughout Wisconsin, Sept., 1948; number tested	1,021
Number from which water was reported unsafe	372
Per cent reported unsafe	36

Since this survey was made, the city has developed deep wells to provide the municipal water supply. Mains have been extended and all parts of the city have access to a safe supply of drinking water which is free from the objectionable features mentioned

It seems clear that the opportunity for dissemination of water-borne diseases was present in the area described. However, the reports of the State Laboratory of Hygiene on analyses of water submitted from private wells throughout Wisconsin during September, 1948, revealed that 372 samples from 1,021 wells were considered "unsafe," 36 per cent. The wells represented varied in depth from less than 25 feet, from which 48 per cent of specimens received were reported "unsafe," to a depth of 450 feet, of which two of twelve wells tested were reported "unsafe." (Table IV.)

Water Supplies, Southwestern Area: The homes of this area obtain water supplies from wells which vary from 55 to 180 feet in depth, the majority over 100 feet deep. Fourteen water supplies were surveyed by the District Sanitary Engineer on Jan. 28, 1949. Most of these were located on farms where cases of infectious hepatitis had occurred. Water from twelve of the fourteen wells was taken for bacteriologic analysis. Of these twelve wells, one was considered entirely satisfactory with respect to location, type of construction, and duration of operation; two were considered probably satisfactory, but the location was poor; the construction of eight others was clearly poor and the location of these ranged from good to poor. The construction of the twelfth well was questionable and the location poor. At the time of sampling, the water from all except one of the wells examined (the last noted) was potable according to *Esch. coli* count standards.

The District Sanitary Engineer concluded from his survey that the wells in this small area of Wiota Township did not reveal significant differences from those in the remaining portion of Lafayette and of adjacent counties. Although many of the wells were improperly constructed and located, the water in most of them was not contaminated at the time the sample was taken. This is a common experience during the winter months when there is little or no flow of surface water. If samples were taken immediately after a wet period, the water in some would be expected to reveal pollution from the surface.

Other Factors.—The food and milk sources for the families throughout the area in the north central section were studied and tabulated. An evaluation of these data failed to reveal sources of contamination or differences between affected and unaffected families. The milk used by homes in the southwestern community was obtained from the private farms. It was unpasteurized. Many of the farmers reported that mastitis was present among the animals in their herds. Examination of the sources of food for the families in the southwestern area failed to reveal suggestive differences.

In neither area were rodents considered a problem; in neither were flies believed to be important.

The survey of each area included a consideration of the numbers and types of livestock and domestic animals present on farms and at homes where cases of infectious hepatitis occurred. The data obtained did not suggest that this factor played a role in the dissemination of infectious hepatitis. However, more information in connection with exact numbers and species of different animals is needed. Study of the nature and character of insect life in the different areas was not made in detail. Data relevant to this aspect should be included in future surveys. A review of the weather reports for the period involved in the two areas was not helpful, so far as could be determined. Tabulation of the incidence of communicable diseases reported to the State Board of Health from the two areas was not suggestive. More detailed examination of these features is desirable.

Serum Transmission: In the city eight children developed the disease after an interval of between 27 to 110 days following immunization procedures in the schools. The other children, who were immunized at the same time, did not develop evidence of the disease. To their knowledge other patients in both areas had not had contact with human products.

COMMENTS

The occurrence of infectious hepatitis in the two localities in Wisconsin described did not constitute an epidemic. In both areas the disease involved a relatively few individuals over a period of several months. Several other observers have described a similar pattern.[1, 4] The seasonal incidence described also has been observed frequently. The disease increased in frequency in the fall of each year and became most prevalent during the winter and early spring months. It did not disappear during the summer.

The age group between 10 and 30 years was most affected, as determined by the criteria employed. Many observers have noted that the disease tends to be mild and often unrecognized in children.[8] This factor may have influenced the results obtained during this survey. The observations demonstrated that there was opportunity for contact transmission of the infection in nearly every case. The opportunity for dissemination of diseases by the gastrointestinal route was also present. In our judgment, the data accumulated during these studies in Wisconsin do not provide conclusive support for any theory so far presented to explain the natural dissemination of this disease.

The most interesting features of this study concern the tendency of infectious hepatitis to occur in families and to be localized geographically without regard to other influences, so far as could be determined. The interval between cases in individual families is another interesting feature. That this interval often consists of several weeks is an observation in common with those of other observers.[1, 9]

Although considerable data were accumulated with respect to livestock, arthropods, domestic animals, weather, geologic features, and other factors concerning the two areas described, more detailed analysis of these data is necessary. Consideration of these various aspects suggests that information of this kind is desirable and should be made the basis for a separate study.

As indicated, the observations which have been made do not seem to fit exactly into any heretofore proposed mechanism for the natural transmission of the disease. It is suggested that other avenues of dissemination, which have not yet been given serious consideration, should be explored. The significance of leptospirosis should be evaluated. Serum transmission of infectious hepatitis as well as serum hepatitis might be an important factor. It does not seem necessary to limit our considerations of the transmission of serum hepatitis to the hypodermic needle. Biting insects might have significance.

Infectious mononucleosis, which often is indistinguishable from infectious hepatitis, both clinically and by laboratory means, has a pattern of occurrence not unlike that of the latter. This should be given consideration. Poliomyelitis presents a pattern of occurrence which, except for seasonal incidence, can be compared with that of infectious hepatitis. It is fair to state that more information about the epidemiology of each of these diseases should be sought by painstaking studies on the ground. There is some likelihood that the solution to the epidemiology of one will be helpful in the efforts to define the mode of dissemination of the others. The information obtained during this study will help to provide a background of knowledge for evaluation of subsequent developments in these areas. Avenues of approach, which may be of importance but which received insufficient attention originally, have already become apparent.

SUMMARY

Observations were made on the epidemiology of infectious hepatitis in two widely separated areas in Wisconsin. The survey revealed that 168 cases in a north central area and 31 cases in a southwestern area of the state had occurred under circumstances which were somewhat similar. The circumstances surrounding the occurrence of these cases were studied. Environmental, geologic, geographic, and other features relating to the areas and individuals affected are reviewed and compared.

The most interesting observations seem to consist of the tendency of the disease to occur and remain localized in families, and to present a limited geographic distribution. The opportunity for air-borne, by direct contact, and water-borne transmission of the disease was present. The evidence obtained did not seem to provide conclusive support for either theory of dissemination.

It is suggested that other methods of transmission, even though unlikely, should be considered in an effort to explain the natural dissemination of infectious hepatitis.

Mr. C. A. Stravinski and Mr. H. L. Lautz, District Sanitary Engineers, made the separate sanitary surveys in each of the areas studied. The State Laboratory of Hygiene, Madison, made the water analyses for the southwestern section. Miss Josephine Foote made the analyses at the Cooperating Laboratory in Wausau. Dr. Francis Cline, District Health Officer, and Dr. A. R. Zintek, Director, Section of Preventable Diseases, State Board of Health, provided assistance.

REFERENCES

1. Paul, J. R., and Gardner, H. T.: Endemiologic Aspects of Hepatitis in U. S. Troops in Germany, 1946-1950, Am. J. Med. 8: 565, 1950.
2. Havens, W. P., Jr.: Infectious Hepatitis, Medicine 27: 279, 1948.
3. Hardy, H. L., and Feemster, R.: Infectious Hepatitis in Massachusetts, With a Review of Present Knowledge of the Disease, New England J. Med. 235: 147, 1946.
4. Neefe, J. R.: Viral Hepatitis: Problems and Progress, Ann Int. Med. 31: 857, 1949.
5. Gellis, S. S., and Janeway, C. A.: Viral Hepatitis, New England J. Med. 239: 503, 1948.
6. Neefe, J. R., Gellis, S. S., and Stokes, J., Jr.: Homologous Serum Hepatitis and Infectious (Epidemic) Hepatitis. Studies in Volunteers Bearing on Immunological and Other Characteristics of the Etiologic Agents, Am. J. Med. 1: 3, 1946.
7. Wiedman, S., and Schultz, A. R.: The Underground and Surface Water Supplies of Wisconsin, Wisconsin Geological and Natural History Review, Bull. XXXV, 1915.
8. Horstmann, D. M., Havens, W. P., Jr., and Deutsch, J.: Infectious Hepatitis in Childhood: A Report of Two Institutional Outbreaks and a Comparison of the Disease in Adults and Children, J. Pediat. 30: 381, 1947.
9. Pickles, W. N.: Epidemiology in Country Practice, Baltimore, 1939, Williams & Wilkins Company.

RADIOACTIVE DI-IODO^{131}-FLUORESCEIN: THE HEALTH PHYSICS ASPECTS OF ITS USE FOR DIAGNOSTIC STUDIES

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THE practical application of iodine^{131} labelled fluorescein in neurosurgery was first demonstrated by Moore[1] in 1947. Following the administration of ordinary (sodium) fluorescein he observed that brain tumor tissue exposed during craniotomy emitted a stronger ultraviolet fluorescence than did normal tissue. After preparing I^{131} labelled fluorescein, Marvin and Moore[2] found that the accumulation of the radiodye in tumor could be detected through the skull by an appropriately placed Geiger-Müller counter. The advantages of such a method were immediately apparent, and a study of the technique was undertaken in the Radioisotope Unit, Veterans Administration Hospital, Hines, Ill. The usefulness of the radiodye method was greatly facilitated by the availability of stable di-iodo^{131}-fluorescein prepared by the Special Research Division, Abbott Laboratories.‡ The results of the successful use of this material in the diagnosis of patients has been reported by Ashkenazy and co-workers,[3] and by Davis, Martin, Ashkenazy, LeRoy, and Fields.[4] It seems likely that other clinical uses for this radiochemical will be developed in the near future. It was obvious at the outset of the study that it was desirable to know the distribution and excretion of di-iodo^{131}-fluorescein so that the radiologic hazard could be evaluated properly. This paper is a report of the studies that have been made on what can be termed the "health physics" aspect of the use of di-iodo^{131}-fluorescein in human subjects.

Any evaluation of a radioisotopic preparation for human use should answer the following questions: (1) What is the distribution of the material in the body? (2) Which tissues or organs have the highest concentration? (3) What is the route and rate of excretion? (4) What amounts of radiation can be anticipated in the tissues of maximum concentration? (5) What is the extent of the radiation hazard to the patient and to his attendants?

If one assumes equal distribution of di-iodo^{131}-fluorescein throughout the body, the "safe tracer dose" can be obtained from Table I of Marinelli, Quimby, and Hine.[5] Considering only the beta radiation, a concentration of 8.3μc/per kilogram will deliver a dose of 0.1 e.r. (equivalent roentgens) to tissue during

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‡The I^{131} for this study was supplied on allocation by the Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tenn.

the first twenty-four hours of exposure. The "safe tracer dose" for a 70 kilogram man would then be 581 μc. This calculation does not take into account either the excretion rate or the selective localization of the isotope in various organs and tissues. Since the customary dose of di-iodo^{131}-fluorescein in neurosurgery is 1,000 to 1,100 μc, it appears that the total radiation hazard to the patient is slight.

MATERIALS AND METHODS

The distribution of di-iodo^{131}-fluorescein was studied in dogs. The dose of radiodye per kilogram was equivalent to that used in human subjects. The methods for radio-assay of tissues, blood, and urine will be described in a later report. Corrections for self-absorption were made, and the over-all accuracy of the methods is better than ± 5 per cent.

RESULTS

The results of a typical experiment are shown in Table I. If 0.0083 μc per gram is the "safe" concentration, it can be seen that such a value is exceeded in every tissue except muscle when samples are obtained seventy-five minutes after injection. The radiodye, however, does not remain long at the site of its initial deposition, and assays made three days after injection show decreasing concentrations in all organs studied except the thyroid gland and the liver. (See Table II.) On the seventh day radioactivity was detected in measurable amounts only in the thyroid gland. The changing concentration in the thyroid gland of dogs is shown in Table III.

TABLE I. DISTRIBUTION OF I^{131} IN A DOG, 75 MINUTES AFTER 185 μC DI-IODO^{131}-FLUORESCEIN, INTRAVENOUSLY

ORGAN	μC/GM.	PER CENT OF DOSE*
Thyroid	0.160	0.216
Lung	0.095	1.240*
Lymph node	0.085	0.830*
Kidney	0.063	4.100
Skin	0.050	53.640*
Liver	0.040	13.830
Brain	0.030	0.580*
Spleen	0.021	2.860
Adrenal	0.009	0.010
Muscle, skeletal	0.003	12.000*
	Total accounted for	89.306

*Where the weight of an organ was not known, it was estimated from the Standard Man table of Lisco.[8]

TABLE II. DISTRIBUTION OF I^{131} IN ORGANS OF THE DOG AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION OF DI-IODO^{131}-FLUORESCEIN (ALL VALUES CORRECTED FOR DECAY TO ZERO TIME)

	PER CENT OF DOSE PER GRAM				
DOG:	1	7	2	3	4
TISSUE TIME:	75 MIN.	3 DAYS	7 DAYS	7 DAYS	7 DAYS
Thyroid	0.086	0.444	0.448	0.059	0.160
Lung	0.051				
Lymph node	0.045		<0.0001		
Kidney	0.034	0.0064	<0.0001		
Skin	0.027				
Liver	0.022	0.330	<0.0001	<0.0001	<0.0001
Spleen	0.021	0.0203	<0.0001		
Brain	0.016	0.0008			
Adrenal	0.0048	0.0011			
Muscle	0.002				

TABLE III. CONCENTRATION OF I^{131} IN THYROID GLAND OF DOGS AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION OF DI-IODO^{131}-FLUORESCEIN (CORRECTED FOR DECAY TO ZERO TIME)

DOG	DOSE (μC)	TIME	μC/GM.	PER CENT DOSE/GM.
1	185	75 min.	0.160	0.086
7	150	3 days	0.666	0.440
8	150	3 days	2.380	1.500
2	185	7 days	0.830	0.448
3	375	7 days	0.220	0.059
4	375	7 days	0.600	0.160

The di-iodo^{131}-fluorescein used in these studies is stable, and no free I^{131} can be detected by ordinary means. It is not possible at this time to report the chemical state of the I^{131} that accumulates in the thyroid gland. We assume, however, that it is not still attached to fluorescein.

Values for concentration of I^{131} in the thyroid similar to those in the dog were observed in patients. The measurements were made with the external Geiger-Müller counter described by Fields and LeRoy,[6] which gives results that are accurate to better than ± 10 per cent. The measurements of euthyroid patients chosen at random are shown in Table IV. Assuming that 1.6 per cent of the dose represents the maximum concentration in the thyroid, and assuming that the average gland weighs 25 grams, the concentration on the first day may be as great as 0.70 μc per gram. This amount of I^{131} will deliver a dose of 8.2 equivalent roentgens. From inspection of Table IV it is seen that the biologic half-life of I^{131} in the thyroid gland is about two to three days. It is obvious

TABLE IV. CONCENTRATION OF I^{131} IN THYROID GLAND OF PATIENTS, MEASURED EXTERNALLY (CORRECTED FOR DECAY TO ZERO TIME)

	TOTAL μC AT VARIOUS TIME INTERVALS (HR.)				
PATIENT	0-6	0-24	24-48	48-72	72-96
1		16.0			
2		8.0			
3			4.0		
4				8.0	3.0
5				1.0	0.5
6					3.5
7	0.5	8.0	4.0		
8*	0.5	2.0	0.5		
9	7.0	4.0	7.0		
10*	7.0	5.0	0.5		
Average	3.7	7.2	3.2	4.0	3.3
Percent of dose	0.370	0.720	0.320	0.40	0.33

*These patients were given 1.0 Gm. KI, orally, just before the administration of the radiodye.

that the radiation to the gland is less than would occur after a minimal tracer dose of 50 μc of NaI^{131}, where the normal uptake is 10 to 40 per cent. It is important to note that this accumulation of I^{131} by the thyroid can be reduced by nonradioactive iodide given by mouth at the time the radiodye is administered.

The concentration of I^{131} remains high in the liver of the dog until the third day, but by the seventh day (Table II) the radioactivity is not measurable. Fluorescein derivatives are excreted in the bile, and the concentration per gram

TABLE V. CONCENTRATION OF I^{131} IN SINGLE SPECIMENS OF DOG BILE, AFTER INTRAVENOUS ADMINISTRATION OF DI-IODO^{131}-FLUORESCEIN (CORRECTED FOR DECAY TO ZERO TIME)

DOG	DOSE (μC)	TIME	μC/GM.	PER CENT DOSE/GM.
5	150	75 min.	1.886	1.250
6	150	210 min.	0.6097	0.400
7	150	3 days	0.0012	0.0008
8	150	3 days	0.0016	0.0011
2	185	7 days	0.00017	0.00009
3	375	7 days	0.0007	0.0004
4	375	7 days	0.0005	0.0003

of gall bladder bile was studied. The results are shown in Table V. Here again, high values were observed shortly after administration of the radiodye. By the third day the concentration was quite low, and by the seventh day it was barely detectable. The total amount of di-iodo^{131}-fluorescein excreted in the bile was not determined in dogs or in man.

TABLE VI. EXCRETION OF I^{131} BY RATS AFTER INTRAPERITONEAL INJECTION OF 25 μC DI-IODO^{131}-FLUORESCEIN (CORRECTED FOR DECAY TO ZERO TIME)

	TOTAL (μC)			
	0 - 24 HR.		24 - 48 HR.	
RAT	URINE	FECES	URINE	FECES
3	1.079	0.102		
4	1.031	0.125		
5	1.731	0.191	0.136	0.015
6	1.481	0.068	0.066	0.004
Average	1.330	0.122	0.101	0.010

In rats the excretion of the di-iodo^{131}-fluorescein in the feces was about 10 per cent of the urinary excretion over a period of forty-eight hours after intraperitoneal injection (Table VI). It seems probable that the same ratio prevails in other mammals. The amount of radiation to which the liver and gastrointestinal tract is exposed does not seem to be excessive or hazardous.

TABLE VII. URINARY EXCRETION OF DI-IODO^{131}-FLUORESCEIN DURING FIRST 24 HOURS AFTER INTRAVENOUS ADMINISTRATION OF 1,000-1,100 μC

CASE	VOLUME/24 HR. (ML.)	I^{131} (μC)	PER CENT OF DOSE/24 HR.	μC/ML. (AVERAGE)
1	1,450	291	29.1	0.20
2	900	121	12.1	0.13
3	1,100	67	6.7	0.06
4	1,800	29	2.9	0.02
5	475	42	4.2	0.09
6	1,325	155	14.1	0.12
6*	2,200	65	5.0	0.03
7	3,525	53	4.8	0.02
8	1,600	160	14.5	0.10
9	1,800	146	13.2	0.08
10	2,200	34	3.4	0.02
10*	1,500	52	5.2	0.03
11	2,900	36	3.6	0.01
12	2,950	43	4.3	0.01
13	2,250	95	9.5	0.04
14	300	79	7.9	0.26
15	825	73	7.3	0.09
Average		90.6	9.0	0.08

*These are repeat estimations, after a second dose of radiodye.

Urinary excretion of di-iodo^{131}-fluorescein is prompt. The amount excreted in the first twenty-four hours by fifteen patients chosen at random varied from 3.2 per cent to 29.1 per cent of the dose; the average was 9.0 per cent (Table VII). Excretion rates on subsequent days are shown in Table VIII. These values for human urine are comparable to the assays of renal tissue from dogs, Table II, where it can be seen that the concentration on the third day is one-fifth of that a seventy-five minutes. It does not appear that the radiodye accumulates in renal tissue to an appreciable extent.

TABLE VIII. DAILY URINARY EXCRETION OF I^{131} AFTER INTRAVENOUS INJECTION OF 1,000 μC DI-IODO^{131}-FLUORESCEIN

TIME (HR.)	TOTAL MICROCURIES* PATIENT				PER CENT†
	SA	FI	AL	RI	
0 – 24	48.2	51.9	61.3	135.2	9.9
24 – 48	6.4	–	5.1	10.8	0.9
48 – 72	3.5	2.5	1.0	3.3	0.4

*All values corrected for decay to zero time.
†Per cent of dose in body at start of time period.

The concentration of di-iodo^{131}-fluorescein in urine is not great enough to constitute a hazard to attendants and nurses handling the urinals, linen, etc., of patients who have received doses of about 1.0 millicurie. The highest concentration observed was 0.26 μc per milliliter for human urine; it was 0.6 μc per milliliter for dog urine (Dog 5 at seventy-five minutes). The average concentration in human urine excreted during the first twenty-four hours was 0.08 μc per milliliter. Solutions of this strength may be discharged directly into the sewer system, according to the Report of the Interim Committee on Waste Disposal, U. S. Atomic Energy Commission.[7] In our laboratory, it is a rule that rubber gloves will be worn when working with solutions containing more than 0.01 μc per milliliter of beta emitters. Attendants caring for incontinent patients usually wash their hands if they are in contact with urine, and as a rule have little occasion to be soiled in this way. Spot-check monitoring of the personnel caring for patients who have received di-iodo^{131}-fluorescein has not shown contamination of the hands. For this reason we have not advised that such attendants wear rubber gloves.

The effective half-life of di-iodo^{131}-fluorescein in the human body can only be estimated from the data available. If we assume that the urinary excretion is 10 per cent on the first day, and the fecal excretion is 10 per cent of the urinary rate, or 1 per cent, and the decay is 8.3 per cent, we find that the loss in the first day is 19.3 per cent of the administered dose. At this rate, the effective half-life would be two to three days. However, serial radiochemical analysis of urine for I^{131} shows a sharp decline in excretion after the tenth hour of the first day. Then, if we examine the average urinary excretion for the second and third days, Table VIII, we find that it is about 1 per cent and 0.5 per cent respectively. Calculating as before, the total loss of I^{131} on the second day would be 1.0 per cent + 0.1 per cent + 8.3 per cent of the I^{131} remaining in the

body, or 9.4 per cent. This is about one-half what one would expect on the basis of the first day's experience. It appears that the clearance of di-iodo^{131}-fluorescein from the body is not a simple affair, and apparently a fraction of the material is "bound" somewhere in the body. Since the excretion is appreciable through the fourth day, it is evident that the effective half-life is less than the physical half-life. It may be considered to be four to seven days for the total body. This is in close agreement with the value found for I^{131} derived from di-iodo^{131}-fluorescein in the thyroid gland. It is interesting to note that with a dose of 1,000 μc, the concentration in the body as a whole may become "safe" (i.e. equal to 580 μc, the amount which will deliver 0.1 e.r.) by the end of the fifth day.

DISCUSSION

The studies that have been described were conducted to determine the distribution and excretion of di-iodo^{131}-fluorescein after intravenous administration. Information was also sought on the effective half-life of the compound and the extent of the radiation hazard created by its use in diagnosis. The results can be summarized as follows.

1. The effective half-life of di-iodo^{131}-fluorescein is not less than four days in man.

2. Significant concentration of the dye is found in the thyroid gland and the liver.

3. The concentration in the thyroid gland is such that a radiation dose of approximately 8.0 e.r. may be delivered to it during the first twenty-four hours. This concentration can be reduced by the proper use of KI.

4. The urinary excretion rate is about 10 per cent on the first day, and about 1 per cent of the balance per day after that. The fecal excretion is assumed to be 10 per cent of the urinary rate.

5. The amount of radiation delivered to the total body in one week can be estimated as 0.8 r, assuming equal distribution, and the beta radiation alone from 390 μc I^{131} disintegrated in the body during that period of time.

6. The urine of patients who receive approximately 1.0 mc. as a test dose can be discharged into the sewer system without special precautions. In the case of incontinent patients, urine passed during the first twenty-four hours may contain amounts of radioactivity sufficiently large to justify the wearing of rubber gloves by attendants. In actual practice, however, no significant contamination of personnel or ward furnishings has been observed.

CONCLUSIONS

Di-iodo^{131}-fluorescein, in doses of 1,000 to 1,100 μc, intravenously, can be used safely for diagnostic purposes.

There is no significant radiation hazard to the patient, or to the institution.

There is a potential radiation hazard in the case of the attendants of incontinent patients, but in practice significant contamination has not been observed.

REFERENCES

1. Moore, G. E.: Fluorescein as an Agent in the Diffentiation of Normal and Malignant Tissues, Science **106**: 130, 1947.
2. Marvin, J. F., and Moore, G. E.: Localization of Brain Tumors With Radiodye: Experimental Evaluation of Physical Limitations, Part II, Nucleonics **3**: 63, 1948.
3. Ashkenazy, Moses, LeRoy, George V., Fields, Theodore, and Davis, Loyal: The Detection of Intracranial Tumors by the Use of Di-iodo^{131}-fluorescein, J. LAB. & CLIN. MED. **34**: 1580, 1949.
4. Davis, Loyal, Martin, John, Ashkenazy, M., LeRoy, George V., and Fields, Theodore: The Clinical Use of Radioactive Di-Iodo^{131}-fluorescein in the Diagnosis and Localization of Tumors of the Central Nervous System, J.A.M.A. In press.
5. Marinelli, L. D., Quimby, E. H., and Hine, S. J.: Dosage Determination With Radioactive Isotopes, Am. J. Roentgenol. **59**: 260, 1948.
6. Fields, Theodore, and LeRoy, G. V.: A Sensitive Geiger-Müller Counter for Determination of I^{131} Content of the Thyroid Gland in Vivo. To be published.
7. Handling Radioactive Wastes in the Atomic Energy Program, U. S. Government Printing Office, Washington, 1949.
8. Lisco, Herman: Standard Man, Argonne National Laboratory Quarterly Report, 1949, ANL-4253, p. 96.

pH OF WHOLE ARTERIAL BLOOD

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ALTHOUGH the glass electrode is an excellent device for measuring the pH of ordinary buffers and laboratory reagents, difficulty has been encountered in adapting it to measure the pH of whole blood. The values for the pH of normal whole blood reported in the literature vary greatly even when measured by the same type of instrument. There are several reasons for this variation. Changes in temperature, conversion of glucose to organic acids following removal of blood from the body, and carbon dioxide exchange between the blood and the atmosphere are all known to alter the pH of the blood.

Effect of Temperature on pH of Blood.—

Rosenthal[1] has suggested that blood pH be determined at room temperature and then corrected to the pH at body temperature. When this method is used, the blood and glass electrode must be at the same temperature. Studies in this laboratory have shown that if a water bath is not used to bring the blood to exactly the same temperature as the electrode, the results are not only variable but grossly inaccurate. The relationship between the pH of blood and the temperature is linear.[1] The effect of temperature on the pH of blood determined in this laboratory is shown in Fig. 1.

Change in pH of Whole Blood After Removal From the Body.—

Harvard and Kerridge[2] found that blood pH decreased with time following withdrawal of blood from the circulation. This is partially caused by continuous glycolysis which increased the hydrogen ions in the blood. Bueding and Goldfarb[3] and Rosenthal[1] found that sodium fluoride retarded but did not completely inhibit blood lactic acid formation and that lowering the temperature of blood markedly decreased the rate of glycolysis. Fig. 2 illustrates the rate of decrease of blood pH at 37.5° C. and at 4° C. as found in this laboratory.

Effect of Gas Exchange With the Atmosphere on the pH of Blood.—

When carbon dioxide is lost in the atmosphere[5] during the pH measurement, the pH of blood is increased. An increase in the oxyhemoglobin of unsaturated blood causes a decrease in the pH of whole blood. For these reasons blood must be removed from the body and be kept under anaerobic conditions.

Simplified Method for Determination of Blood pH With a Glass Electrode.—

In order to simplify and render the measurement of blood pH accurate for routine clinical purposes, the following procedures have been developed. The

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glass electrode assembly* was placed in a constant-temperature cabinet† that was controlled by a mercury to mercury thermoregulator.‡ All electrical circuits were shielded and the apparatus was grounded adequately. An inner

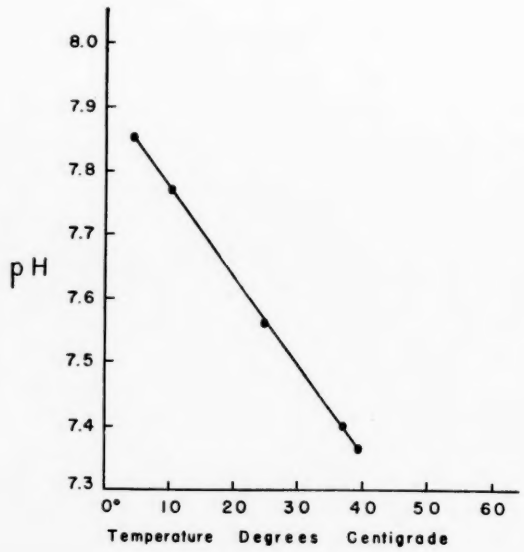

Fig. 1.—The relationship of the pH of a sample of whole blood to temperature.

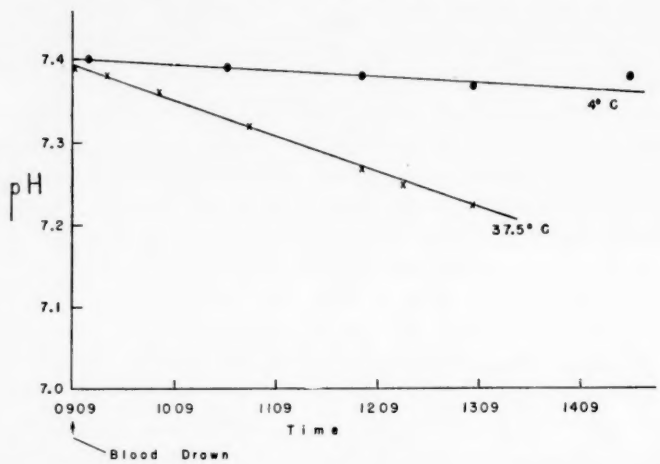

Fig. 2.—The change of the pH of whole blood after removal from the body. The upper curve is that of blood kept at 4° C. The lower curve is that of blood kept at 37.5° C.

plastic door with two working entrances covered by pliable plastic material was installed to maintain the constant temperature. Within the cabinet (Fig. 3) is sufficient space for all buffer solutions, glass electrode, and saturated potassium

*Cambridge Instrument Company, New York, N. Y.
†Modern Electric Laboratory, Chicago, Ill.
‡Precision Scientific Company, Chicago, Ill.

chloride solution. Once the proper equipment is installed and buffer solutions are prepared meticulously, the determination of the pH of whole blood becomes a relatively simple and practical procedure.

Three buffer solutions were prepared. The standard buffer was prepared by dissolving 0.05 mol of United States Bureau of Standards potassium acid phthalate* in 1 liter of boiled distilled water. This solution has a pH of 4.025 at 37.5° C. and was used to standardize the stock buffer solutions. Stock buffer

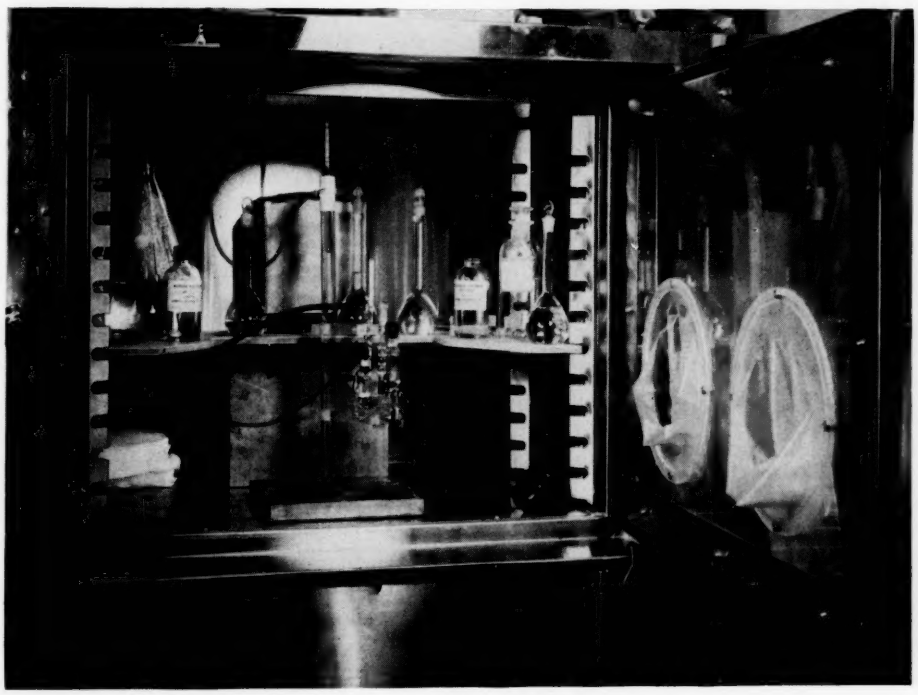

Fig. 3.—The glass electrode in the constant-temperature cabinet.

solution No. 1 was prepared by placing 0.03 mol C.P. sodium hydrogen phosphate, 0.02 mol C.P. potassium dihydrogen phosphate, and 0.02 mol sodium chloride in 1 liter of boiled distilled water. This solution has a pH of 6.970 at 37.5° C. Stock buffer solution No. 2 was prepared in the same manner as the standard buffer solution except that chemically pure (ACS) potassium acid phthalate was used instead of that obtained from the United States Bureau of Standards.

The pH meter was standardized with both stock buffer solutions before measuring the pH of each sample, and the pH of both stock buffer solutions was frequently checked against the United States Bureau of Standards buffer solution. The glass electrode was cleaned with distilled water after each determination. At the end of each day the electrode was cleaned with a saturated solution of urea in water.

*This buffer material may be purchased from the U. S. Bureau of Standards, Washington, D. C.

Blood samples were withdrawn anaerobically in oiled syringes containing 0.2 c.c. of heparin to prevent coagulation. The tip of the syringe was sealed with a glass plug inserted into a rubber tube 2 cm. in length in order to prevent gas exchange between the blood and the atmosphere. The syringes were maintained at 37.5° C. before and after collection of the blood samples with a hot water bottle.

Arterial blood was removed from normal human beings and the whole blood pH determined at 37.5° C. The mean pH of eleven normal subjects was 7.401 ± 0.003.*

Although it is preferable to determine the blood pH within a few minutes after removal from the body, a method involving extrapolation can be used if a brief delay is unavoidable. If the temperature remains constant the pH of whole blood decreases with time in a linear relationship (Fig. 2). In applying this method the 10 c.c. syringe containing the blood is immediately placed in the constant-temperature cabinet at 37.5° C. Multiple determinations of pH are made and the values are plotted against the time on linear graph paper. The blood pH can then be estimated by extrapolating to zero time. Because of the rapid change in blood pH the first determination of pH should be done within twenty minutes of removal of blood from the body. The mean pH of the blood of eighteen normal subjects determined by the extrapolation method was 7.408 ± 0.018.

CONCLUSIONS

The pH of whole blood can be measured accurately with the glass electrode. The temperatures of the blood and glass electrode must be the same during the determination. Measurements of the pH should be done immediately at 37.5° C., or if this is not convenient an extrapolation method may be used. A constant-temperature cabinet has been described for maintaining the glass electrode and buffer solution at 37.5° C.

REFERENCES

1. Rosenthal, T. B.: The Effect of Temperature on the pH of Blood and Plasma in Vitro, J. Biol. Chem. **173**: 25, 1948.
2. Harvard, R. E., and Kerridge, P. T.: An Immediate Acid Change in Shed Blood, Biochem. J. **23**: 600, 1929.
3. Bueding, E., and Goldfarb, W.: The Effect of Sodium Fluoride and Sodium Iodoacetate on Glycolysis in Human Blood, J. Biol. Chem. **141**: 539, 1941.
4. Stadie, Wm., O'Brien, H., and Laug, E.: Determination of the pH of Serum at 38° With the Glass Electrode and an Improved Electron Tube Potentiometer, J. Biol. Chem. **91**: 243, 1931.
5. Parsons, T. R. P.: On the Reaction of the Blood in the Body, J. Physiol. **51**: 440, 1917.
6. Bates, R. G., and Acree, F.: pH Value of Certain Phosphate-Chloride Mixtures and the Second Dissociation Constant of Phosphoric Acid from Zero Degrees to 60 Degrees Centigrade, Journal of Research of the National Bureau of Standards **30**: 129, 1943.
7. Hamer, W. J., Pinching, G. D., and Acree, S. F.: pH Standards at Various Temperatures: Aqueous Solution of Acid Potassium Phthalate, Journal of Research of the National Bureau of Standards **36**: 47, 1946.

*Standard deviation.

THE EFFECT OF CORTISONE IN GLOMERULONEPHRITIS AND THE NEPHROPATHY OF DISSEMINATED LUPUS ERYTHEMATOSUS

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THE dramatic response of some of the hypersensitivity diseases to pituitary adrenocorticotrophin and cortisone suggested that these hormones might favorably affect the course of glomerulonephritis and the nephropathy of disseminated lupus erythematosus. Although cortisone has failed to prevent cytotoxic serum nephritis in rats,[1] the reports by Farnsworth[2, 3, 4] on the treatment of clinical glomerulonephritis with ACTH have been encouraging. Thorn and associates,[5] on the other hand, have not been able to confirm these encouraging results.

While ACTH and cortisone may have a strikingly beneficial effect on many features of disseminated lupus erythematosus,[5-10] their action on the renal manifestations of this disease are not well documented. In the six cases of disseminated lupus erythematosus treated with ACTH by Thorn and colleagues,[5] all had evidence of renal involvement. These authors report complete resolution of the renal lesion in one patient and a reduction in azotemia, when present, in all but one subject.

On the basis of theoretical considerations and in view of encouraging preliminary reports, it was considered advisable to study carefully the effects of cortisone on renal function in patients with renal disease. The data presented in this report are derived from the investigation of four patients in various phases of glomerulonephritis and two patients with the nephropathy of disseminated lupus erythematosus.

METHODS

In each patient control observations were made prior to the institution of cortisone therapy. Detailed observations were conducted during the time of treatment and for a variable period thereafter. Although rigid metabolic studies were not carried out, each patient received a constant diet during the investigation, and a saline suspension of cortisone acetate (Merck) was given intramuscularly in divided doses.

Renal function was evaluated by the use of the usual clinical studies and by the clearance and saturation methods for measuring hemodynamics. Twenty-four hour urine collection periods were established, and daily routine urinalyses were made. The twenty-four hour urine albumin and the blood urea nitrogen were determined twice weekly in each patient. The effective renal plasma flow and the maximal tubular excretory capacity were measured by the use of sodium para-aminohippurate, and the glomerular filtration rate was measured by the clearance of mannitol.[11] Sodium and potassium determinations were performed with the Perkin-Elmer internal standard flame photometer.

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CASE REPORTS AND RESULTS

Case 1 (A. F.): Acute Diffuse Glomerulonephritis.—A 30-year-old man was admitted to the hospital April 23, 1950, complaining of abdominal pain and arthralgia. He had had a severe sore throat three weeks previously.

The fundoscopic examination was negative. The blood pressure was 106 mm. Hg systolic and 72 mm. diastolic. The erythrocyte sedimentation rate was 75 mm. per hour. The urinalysis was negative. On the eleventh hospital day the patient developed classical acute glomerulonephritis manifested by hypertension, periorbital and peripheral edema, oliguria, albuminuria, hematuria, and azotemia. After a five-day control period the patient was started on cortisone. He received 200 mg. daily for twenty-three days. Despite his claim that he felt better, his appetite did not improve, and he lost 5 pounds in weight. This weight loss, however, was associated with progressive edema and the development of ascites, and pleural effusion. Although weight loss continued after the end of therapy, the clinical picture of anasarca developed.

The blood pressure was moderately elevated during the control period (150/100) and then fell gradually to normal by the third treatment week. With therapy the patient developed a polymorphonuclear leucocytosis and an eosinopenia. The sedimentation rate remained elevated. Initially the serum proteins were 5.5 Gm. per cent with 3.3 Gm. albumin and 2.2 Gm. globulin. Associated with massive albuminuria total proteins fell to 3.3 Gm. per cent with 1.1 Gm. albumin and 2.4 Gm. globulin. Hypercholesterolemia developed during the second week of cortisone administration when other evidences of the nephrotic syndrome were manifest. Sodium retention and increased potassium excretion occurred with treatment, but sodium and water diuresis did not ensue when the drug was stopped. Observations concerning renal hemodynamics, blood urea nitrogen, and quantitative determinations of albuminuria and hematuria are given in Table I.

TABLE I. CORTISONE IN ACUTE GLOMERULONEPHRITIS—PATIENT A. F.

	R.P.F. (ML. PER MIN.)	G.F. (ML. PER MIN.)	F.F. (%)	URINE RED CELLS	URINE ALBUMIN (GM./24 HR.)	BLOOD UREA NITROGEN (MG. %)
Control						
Week 1	511	44.4	11.5	4+	14.9	52.8
Treatment						
Week 1	563	35.3	6.3	4+	17.2	55.4
Week 2	612	31.2	5.2	4+	22.2	54
Week 3	693	49.7	7.2	4+	15.2	46.5
Week 4	651	48.2	7.4	4+	---	50
Post treatment						
Week 1	363	38.5	10.6	4+	14.8	41.5
Week 2	423	32.5	7.6	4+	13.4	27.8

R.P.F., renal plasma flow.
G.F., glomerular filtration rate.
F.F., filtration fraction.
1+ = 3 to 10 red blood cells per high-power field.
2+ = 10 to 30 red blood cells per high-power field.
3+ = 30 to 100 red blood cells per high-power field.
4+ = More than 100 red blood cells per high-power field.

Summary: A patient with severe, acute glomerulonephritis was treated with 4.65 Gm. cortisone during a twenty-three day period. The course of the disease was unaltered. Notwithstanding therapy, the nephrotic syndrome developed.

Case 2 (M. K.): Acute Diffuse Glomerulonephritis.—A 58-year-old man was admitted to the hospital Dec. 14, 1949, with streptococcic pneumonia, empyema, and pericarditis. The blood pressure was normal, and the fundoscopic examination was negative. On the eleventh

hospital day he developed classical acute glomerulonephritis with hypertension, pulmonary edema, ankle edema, albuminuria, hematuria, and azotemia. During the course of this disease hemorrhages and exudates were observed in the fundi. The pulmonary and ankle edema resolved, and the hypertension decreased. The patient was improving spontaneously. However, since he still showed evidence of renal dysfunction, hematuria, albuminuria, and hypertension he was selected for cortisone therapy. For the purpose of this investigation he was maintained on a diet containing 200 mg. of sodium daily.

Control observations were carried out for seven days. Treatment consisted of 100 mg. cortisone daily for twenty-one days and 200 mg. daily for seven days. With hormone administration there was marked improvement in the feeling of well-being and increased appetite. Moderate euphoria was apparent. He developed a marked "moon" face. The weight increased progressively during treatment and continued to increase after the drug was discontinued. This was not associated with edema formation. A moderate hypertension (170/90) persisted during the first course of therapy. Subsequently, there was a slight drop in the systolic and diastolic pressures (158/84).

A neutrophilic leucocytosis and an eosinopenia were noted during treatment. The sedimentation rate decreased gradually from 102 mm. per hour to 60 mm. per hour and continued to fall to 27 mm. per hour after the drug was stopped. The initial hemoglobin was 7.1 Gm. per cent and it rose gradually to 11.8 Gm. per cent at the end of the investigation. The patient had mild diabetes mellitus which was unaffected by cortisone administration. There was no significant change in the serum proteins or the serum cholesterol.

Cortisone caused sodium retention and moderate potassium diuresis. Subsequent to treatment there was water diuresis without concomitant sodium diuresis. Quantitative determinations of renal plasma flow, glomerular filtration rate, blood urea nitrogen, albuminuria, and hematuria are recorded in Table II. Six weeks after the second treatment period the routine urinalysis was negative except for a trace of albumin. The Addis count, however, still demonstrated marked hematuria and cylindruria.

TABLE II. CORTISONE IN ACUTE GLOMERULONEPHRITIS—PATIENT M. K.

	R.P.F. (ML. PER MIN.)	G.F. (ML. PER MIN.)	F.F. (%)	URINE: RED CELLS	URINE: ALBUMIN (GM./24 HR.)	BLOOD UREA NITROGEN (MG. %)
Control						
Week 1	186	26.5	14.1	4+	0.65	27.5
Treatment						
Week 1	204	35.7	17.5	4+	0.79	26
Week 2	221	42.6	18.7	4+	0.37	23
Week 3	222	42.3	19.0	4+	0.53	22
Post treatment						
Week 1	263	56.0	21.0	4+	0.41	23
Week 2	240	45.0	18.7	4+	0.57	22
Treatment						
Week 1	239	64.0	22.0	2+	0.30	22
Post treatment						
Week 1	243	53.3	21.9	3+	0.57	23
Week 2	261	50.8	22.2	4+	0.30	22
Week 3	214	43.8	20.4	--	---	---

Summary: A patient with acute glomerulonephritis was treated with 3.5 Gm. of cortisone for twenty-eight days. Spontaneous improvement in azotemia and albuminuria preceded therapy. There was no evidence to indicate that cortisone resulted in further improvement.

Case 3 (L. L.): Subacute Glomerulonephritis.—A 30-year-old man was admitted to the hospital on Feb. 10, 1950. Two weeks after a severe upper respiratory infection in December, 1949, he had general malaise and periorbital and ankle edema. In January, 1950, he was found to have hypertension, hematuria, and albuminuria. The initial blood pressure was

144 mm. Hg systolic and 92 diastolic. The sedimentation rate was 23 mm. per hour. He had persistent hematuria and albuminuria. There was 10 per cent excretion of phenolsulfonphthalein in thirty minutes.

The patient was given a diet containing 800 mg. sodium per day. After a two-week control period cortisone was administered in a dose of 150 mg. daily for twenty-eight days. The appetite was considerably improved, and he "felt better than he had for months." Mild euphoria, a conspicuous "moon" face, and acne were noted. He complained of dyspepsia early in the course of therapy. The weight increased 4 pounds and returned rapidly to normal in the post-treatment period. Edema was not present, and the arterial blood pressure was not altered by treatment.

The polymorphonuclear leucocyte and eosinophil response were similar to those in the two preceding patients. The sedimentation rate was slightly elevated during the control phase (22 mm. per hour). There was marked rise during the first ten days of treatment (79 mm. per hour). Subsequently, the sedimentation rate fell to normal (5 mm. per hour). There was no change in the serum proteins. Associated with treatment there was a drop in the serum cholesterol, due almost exclusively to depression of the cholesterol esters.

There was no apparent retention of water or sodium during hormone administration. Urinary potassium excretion increased slightly. After the conclusion of treatment marked sodium diuresis occurred. In Table III are recorded the renal hemodynamic measurements, blood urea nitrogen, albuminuria, and hematuria. Subsequent observations revealed that the glomerular filtration rate had returned to the control value.

TABLE III. CORTISONE IN SUBACUTE GLOMERULONEPHRITIS—PATIENT L. L.

	R.P.F. (ML. PER MIN.)	G.F. (ML. PER MIN.)	F.F. (%)	URINE RED CELLS	URINE ALBUMIN (GM./24 HR.)	BLOOD UREA NITROGEN (MG. %)
Control						
Week 1	684	79.4	11.6	3+	7.4	22.5
Week 2	650	80.9	12.4	3+	5.5	16.4
Treatment						
Week 1	694	89.2	12.9	3+	8.7	18.5
Week 2	704	98.3	13.9	3+	7.9	20.7
Week 3				2+	7.1	18.5
Week 4	644	100.9	15.7	2+	6.5	16.0
Post treatment						
Week 1	658	100.8	15.3	3+	4.6	16.7
Week 2	674	108.8	16.1	4+	6.4	15.0
Week 5	597	75.3	12.6	--	---	---

Summary: A patient with subacute glomerulonephritis was treated in the fifth month of the disease with 4.25 Gm. cortisone during a twenty-eight day period. The glomerular filtration rate, urea clearance, and phenolsulfonphthalein excretion increased. However, failure of regression of hematuria and albuminuria indicated persistent activity of the basic pathologic process.

Case 4 (P. S.): Chronic Glomerulonephritis, Nephrotic Phase.—A 41-year-old man was admitted to the hospital on Feb. 13, 1950. In July, 1948, he first noted ankle edema, oliguria, and smoky-colored urine. Thereafter, he had recurrent ascites, edema, malaise, and weakness. Fluctuating hypertension, hypoproteinemia, hematuria, and albuminuria had been noted. On this admission the blood pressure was 160 mm. Hg systolic and 100 diastolic. There was mild arteriovenous nicking in the fundi. Ascites and peripheral edema were present. The hemoglobin was 11.4 Gm. per 100 c.c., and the erythrocyte sedimentation rate was 64 mm. per hour. Albuminuria and hematuria were present. Shortly after admission abdominal paracentesis was performed with the removal of 6,000 c.c. of fluid. The peripheral edema disappeared.

The patient received a diet containing 400 mg. of sodium per day. Following an eight-day period of control studies, he was treated with 100 mg. of cortisone daily for twenty-one days. After a two-week interval he was re-treated with 200 mg. daily for nine days. The appetite improved markedly, and the patient desired more food despite a daily intake of 4,100 calories. Euphoria with hypomania became pronounced. A definite "moon" face and purple abdominal striae appeared. He complained of transient dyspepsia and vague muscular cramps. There was a weight gain of 14 pounds during treatment, and no weight loss after treatment was discontinued. Ascites appeared after fifteen days and peripheral edema after nineteen days of therapy. The average blood pressure was 140 mm. Hg systolic and 86 diastolic during the control phase. With treatment hypertension developed (170/96). Ten days after therapy was stopped, the blood pressure was normal.

Marked leucocytosis and eosinopenia appeared. The sedimentation rate was unchanged during the first treatment period and actually rose during the second period. The serum proteins were 5.2 Gm. per cent with 2.5 Gm. albumin and 2.7 Gm. globulin. This was unchanged by therapy. The serum cholesterol of 262 mg. per cent was not altered by cortisone. After the drug was stopped, hypercholesterolemia occurred (334 mg. per cent).

Sodium retention and moderate potassium diuresis occurred during treatment. There was moderate diuresis of sodium chloride, and of water in the post-treatment phase. In spite of this, edema persisted. The determinations of renal hemodynamics, blood urea nitrogen, albuminuria, and hematuria are given in Table IV.

TABLE IV. CORTISONE IN CHRONIC GLOMERULONEPHRITIS—PATIENT P. S.

	R.P.F. (ML. PER MIN.)	G.F. (ML. PER MIN.)	F.F. (%)	URINE RED CELLS	URINE ALBUMIN (GM./24 HR.)	BLOOD UREA NITROGEN (MG. %)
Control						
Week 1	344	63.0	18.4	3+	2.54	16
Treatment						
Week 1	318	53.0	16.7	4+	5.10	16
Week 2	439	63.0	14.8	4+	5.30	20
Week 3	394	57.0	14.4	4+	6.60	27
Post treatment						
Week 1	446	73.0	16.3	4+	5.88	25
Week 2	394	61.0	15.5	4+	3.73	16
Treatment						
Week 1	384	68.0	17.5	3+	3.73	17
	419	64.0	15.3			
Post treatment						
Week 1	439	67.8	15.4	4+	8.10	23
Week 2	356	56.2	15.8	3+	5.29	17
Week 3	---	---	---	2+	3.70	13

Summary: A patient with the nephrotic phase of chronic glomerulonephritis was treated for a thirty-day period with 3.8 Gm. of cortisone. There was no objective evidence that the course of the disease was altered.

Case 5 (D. O.): Chronic Disseminated Lupus Erythematosus.—A 28-year-old white woman was admitted to the hospital on Jan. 17, 1950, with classical disseminated lupus erythematosus. Fever, arthralgia, cutaneous eruption, and pleural effusion had been present intermittently for two years. Physical examination revealed the typical skin lesion of this disease. There was a bilateral pleural effusion. The blood pressure was 180 mm. mercury systolic and 105 diastolic. The arterial tension gradually decreased, but mild hypertension prevailed during the control period of observation. A moderate hypochromic anemia and leucopenia were present. The erythrocyte sedimentation rate was 120 mm. in one hour. The urinalysis revealed marked albuminuria and hematuria. A skin biopsy confirmed the diagnosis of disseminated lupus erythematosus.

Control observations were made for two weeks. The diet contained 1,400 mg. of sodium daily. A total of 4.625 Gm. of cortisone was administered during a twenty-eight day period. Shortly after the induction of therapy the temperature became normal. The skin lesions had cleared markedly prior to our investigations; however, a prominent pigmentation persisted. During cortisone administration there was almost complete fading of this pigmentation. Marked subjective improvement, a "moon" face, and acne were noted.

The weight increased 14 pounds, and peripheral edema became apparent during treatment. The weight then returned to the control levels within fourteen days after the drug was stopped. There was a striking increase in the blood pressure (214/110 during the third week of hormone administration. This degree of hypertension persisted for two weeks after the cortisone was discontinued.

A mild leucocytosis and eosinopenia developed. The erythrocyte sedimentation rate decreased gradually from 118 mm. per hour to 18 mm. per hour during treatment. During hormone administration there was sodium retention and potassium diuresis. In the post-treatment phase marked sodium and water diuresis occurred. The measurements of renal function, blood urea nitrogen, albuminuria, and hematuria are recorded in Table V.

TABLE V. CORTISONE IN LUPUS ERYTHEMATOSUS—PATIENT D. O.

	R.P.F. (ML. PER MIN.)	G.F. (ML. PER MIN.)	F.F. (%)	URINE: RED CELLS	URINE: ALBUMIN (GM./24 HR.)	BLOOD UREA NITROGEN (MG. %)
Control						
Week 1	434	45.0	10.4	2+	0.59	23.7
Week 2	428	48.4	11.3	1+	0.45	20.0
Treatment						
Week 1	---	---	---	2+	0.73	18.7
Week 2	396	57.0	14.4	2+	0.60	19.3
Week 3	376	60.8	16.2	2+	1.10	19.5
Week 4	354	62.5	17.7	3+	2.50	17.7
Post treatment						
Week 1	309	---	---	4+	4.45	13.3
Week 2	371	73.0	19.7	3+	4.25	13.5
Week 3	394	52.7	13.4	2+	3.53	16.5

Summary: A patient with chronic disseminated lupus erythematosus with renal involvement received 4.625 Gm. of cortisone during a twenty-eight day period. A severe hypertension developed, and hematuria and albuminuria increased.

Case 6 (R. J.): Subacute Disseminated Lupus Erythematosus.—A 23-year-old man was admitted to the hospital May 22, 1950, complaining of a skin eruption, swelling of the ankles, and arthralgia. A diagnosis of disseminated lupus erythematosus had previously been made and was confirmed clinically and histologically in this hospital. Physical examination revealed an acutely ill man. There was a diffuse erythematous lesion involving the forehead, face, and ears. Periorbital edema was present. Superficial ulcerations of the lips and hard palate were seen. There was a bilateral pleural effusion. The blood pressure was 140 mm. mercury systolic and 84 diastolic. There was pitting edema of the extremities and edema of the genitalia. Generalized lymphadenopathy was noted. A moderate hypochromic anemia and leucopenia were present. The erythrocyte sedimentation rate was 61 mm. in one hour. The urinalysis revealed albuminuria, hematuria, and cylindruria. The serum proteins were 3.5 Gm. per 100 c.c. with 1.8 Gm. per cent albumin and 1.7 Gm. per cent globulin.

The patient was given a constant diet containing 200 mg. sodium daily. Control observations were made for two weeks. Cortisone was administered in a dose of 150 mg. daily for twenty-five days. Prior to therapy the patient had intermittent fever as high as 101.4° F. Within forty-eight hours after the induction of hormone administration the temperaure became normal; but two weeks after cortisone treatment was stopped, fever recurred. There was

complete healing of the oral ulcerations within three days after the start of cortisone. The cutaneous lesions showed definite improvement within thirty-six hours and continued to regress throughout the course of therapy. The generalized lymphadenopathy resolved slowly. A six-pound weight gain occurred during treatment and was associated with the development of ascites and an increase in peripheral edema. This was followed by a weight loss of sixteen pounds in the post-treatment period, but the ascites and edema persisted. Only a small part of this weight loss could be attributed to salt and water diuresis. The blood pressure increased to 180 mm. mercury systolic and 114 diastolic during therapy, and a marked hypertension has persisted.

The erythrocyte sedimentation rate decreased from 106 mm. in one hour to 63 and then rose above the control level in the post-treatment period. The serum proteins decreased to 3.1 Gm. per 100 cubic centimeters. Moderate leucocytosis and eosinopenia occurred. Evidence of mild sodium retention and potassium diuresis was observed during cortisone administration. Despite this the serum sodium decreased progressively to 120.9 meq. per liter and the serum potassium rose to 8.4 meq. per liter. Three weeks after the drug was stopped, these serum electrolyte concentrations were again normal. The values for the renal plasma flow, glomerular filtration rate, blood urea nitrogen, albuminuria, and hematuria are recorded in Table VI.

TABLE VI. CORTISONE IN LUPUS ERYTHEMATOSUS—PATIENT R. J.

	R.P.F. (ML. PER MIN.)	G.F. (ML. PER MIN.)	F.F. (%)	TM_{pah}* (MG./MIN.)	URINE RED CELLS	URINE ALBUMIN (GM./24 HR.)	BLOOD UREA NITROGEN (MG. %)
Control							
Week 1	688	46.9	6.8	---	2+	9.0	43.5
Week 2	777	42.3	5.4	67.5	3+	9.5	53.0
Treatment							
Week 1	---	---	---	---	3+	11.5	66.8
Week 2	---	---	---	---	3+	11.4	66.0
Week 3	572	27.9	4.9	---	4+	12.7	77.5
Week 4	584	30.7	5.3	30.8	4+	12.8	65.0
Post treatment							
Week 1	---	---	---	---	3+	---	---
Week 2	493	28.7	5.8	---	4+	15.3	57.5
Week 3	---	---	---	---	4+	19.0	48.0

*Tm_{pah} = maximal tubular excretory capacity for sodium para-aminohippurate.

Summary: A patient with subacute disseminated lupus erythematosus with severe renal involvement was treated with 3.75 Gm. of cortisone during a twenty-five day period. Although other manifestations of the disease were altered, the renal lesion was unaffected. Progressive renal insufficiency with the nephrotic syndrome and severe hypertension developed.

DISCUSSION

The functional alterations of the kidney in the various phases of glomerulonephritis have been well defined by Earle, Taggart, and Shannon.[12] Earle[13] has also observed the modification of renal hemodynamics in two cases of disseminated lupus erythematosus. The low filtration fraction, resulting from a disproportionate reduction in the glomerular filtration rate, relative to the renal plasma flow, is illustrated in the control observations of three patients with acute and subacute glomerulonephritis and two patients with disseminated lupus erythematosus. The patient with the nephrotic phase of chronic glomerulonephritis had a normal filtration fraction. Analysis of the data

(Tables I to VI) reveals that transient improvement in renal function, as measured by the renal plasma flow and glomerular filtration rate, may occur during the administration of cortisone. It is apparent that some of the observed changes are due to spontaneous fluctuations in the disease process and that others are associated with cortisone therapy. The pattern of the observed responses, with a return of renal function to control levels in most instances, indicates an association with the administration of cortisone.

The mechanism by which transient increases in renal function may be elicited by cortisone is unknown. It has already been indicated that the administration of cortisone must, in some way, be related to the observed changes. Two mechanisms which may be involved are as follows: (1) A direct effect of cortisone upon the glomerular capillary bed and renal vasculature, (2) an indirect effect of cortisone mediated through an increased extracellular fluid volume.

The first hypothesis is not feasible since hematuria and albuminuria persisted during cortisone treatment. Improvement in renal function due to a direct action of cortisone on the glomerular capillaries, without change in the degree of hematuria and albuminuria, would be most paradoxical. The possible effect of cortisone upon the renal arterioles cannot be evaluated at present. The second hypothesis may be involved in the explanation of some of the observed changes. Experimental and clinical observations have established the fact that adrenal cortical hormones can alter renal function. That a deficiency of adrenal cortical hormones is associated with decreased renal plasma flow and glomerular filtration rate is well established. Desoxycorticosterone acetate will increase the diminished glomerular filtration rate,[14] and aqueous adrenal cortical extract will cause a further rise, but not to normal levels.[15] The action of DOCA is mediated through an increase in the extracellular fluid volume.[16] This increase results in elevation of both the renal plasma flow and glomerular filtration rate. The mode of action of adrenal cortical extract, in a normally hydrated patient with Addison's disease, is unknown. In short-term experiments in normal man, cortisone has been found to increase the renal plasma flow without affecting the glomerular filtration rate.[17] The Tm_{pah} may be increased. Pituitary adrenocorticotrophin increased both glomerular filtration rate and renal plasma flow. It is entirely conceivable that the diseased kidney may respond in a similar manner to an expanded extracellular fluid volume. Thus cortisone could cause transient changes in renal function without altering the basic disease process.

Perera and associates[18, 19] report that in patients with hypertensive vascular disease cortisone has a depressor effect on "resting" blood pressure. ACTH, however, was noted to cause a rise in the arterial pressure in both normotensive and hypertensive subjects.[20] Thorn[5] and Perera[20] suggest that the effect of ACTH may be due to pressor steroids from the adrenal cortex. Sprague and colleagues[21] postulate that the presence of renal disease may alter the effect of ACTH and cortisone on the arterial tension. In disseminated lupus erythematosus with renal involvement cortisone may cause a

striking rise in the blood pressure.[21] In experimental nephritis in rats this hormone was noted to cause a moderate rise in the arterial pressure.[1] Adrenalectomy markedly accentuated this hypertensive effect. In patients treated with ACTH, Thorn[5] reports a rise in blood pressure in three of nine subjects with chronic glomerulonephritis and no change in three subjects with the nephrotic syndrome. In our experience with cortisone the blood pressure was noted to fall rather promptly in one patient with acute glomerulonephritis and to decline gradually in the other patient with acute glomerulonephritis. These changes may have developed spontaneously in the natural course of the disease. There was no change in the arterial pressure in the patient with subacute glomerulonephritis. Moderate hypertension developed in the patient with the nephrotic phase of chronic glomerulonephritis, and severe hypertension developed in both patients with disseminated lupus erythematosus. While cortisone does not usually affect the blood pressure,[21] it may, under certain circumstances, produce marked hypertension. Renal disease may be indirectly implicated in the mechanism responsible for the pressor action of cortisone, but the exact mechanism is unknown.

One of the most interesting observations of this study was the effect of cortisone on the serum potassium in one patient with moderate renal insufficiency and a markedly reduced filtration rate. During therapy the potassium rose progressively and then fell to normal after cessation of hormone treatment. Reciprocal changes in the serum sodium concentration were observed. This same phenomenon was noted when the patient was treated again for eight days with cortisone. The dietary intake remained constant during these periods of observation. It has previously been shown that cortisone causes potassium diuresis and with sufficient doses may cause hypopotassemic alkalosis.[21] This would suggest that cortisone has a direct action on the renal tubules and interferes with reabsorption of the potassium ion. Sprague and associates[21] have, however, suggested that some of the increased urinary potassium may be derived from the intracellular fluid. Our findings demonstrate that cortisone may have a direct action on the cellular membrane with a liberation of intracellular potassium. Such an assumption seems necessary to explain the otherwise paradoxical rise in serum potassium during mild potassium diuresis. Whether increase cellular permeability occurs more readily in disseminated lupus erythematosus is unknown at present. With normal renal function the liberated potassium is entirely excreted in the urine, but with renal insufficiency the electrolyte changes may be manifested by a rise in the serum potassium concentration. The associated fall in the serum sodium concentration at a time of sodium retention may be due to either a disproportionately greater retention of water or to a shift of sodium into the cell.

SUMMARY

Four patients in the various phases of glomerulonephritis and two patients with the nephropathy of disseminated lupus erythematosus were treated with cortisone. Although transient changes in renal function were observed, hematuria and albuminuria were unabated and, in some instances, actually in-

creased. Thus, cortisone had no beneficial effect upon the basic pathologic process in the glomerular capillaries. The mechanism by which cortisone may mediate alterations in renal function is discussed.

REFERENCES

1. Knowlton, A. I., Loeb, E. N., Stoerk, H. C., and Seegal, B. C.: The Development of Hypertension and Nephritis in Normal and Adrenalectomized Rats Treated With Cortisone, Proc. Soc. Exper. Biol. & Med. **72**: 722, 1949.
2. Farnsworth, E. B.: Studies on the Influence of Adrenocorticotropin in Acute Nephritis, in Simple Nephrosis and in Nephrosis With Azotemia, Proceedings of the First Clinical ACTH Conference, edited by J. R. Mote, Philadelphia, 1950, The Blakiston Company, pp. 297-317.
3. Farnsworth, E. B.: Acute and Subacute Glomerulonephritis Modified by Adrenocorticotropin, Proc. Soc. Exper. Biol. & Med. **74**: 57, 1950.
4. Farnsworth, E. B.: Metabolic Changes Associated With Administration of Adrenocorticotropin in the Nephrotic Syndrome, Proc. Soc. Exper. Biol. & Med. **74**: 60, 1950.
5. Thorn, G. W., Forsham, P. H., Frawley, T. F., Hill, S. R., Jr., Roche, M., Staehlin, D., and Wilson, D. L.: The Clinical Usefulness of ACTH and Cortisone, New England J. Med. **242**: 783, 865, 1950.
6. Baehr, G., and Soffer, L. J.: Treatment of Disseminated Lupus Erythematosus With Cortisone and Adrenocorticotropin, Bull. New York Acad. Med. **26**: 229, 1950.
7. Thorn, G. W., Bayles, T. B., Massell, B. F., Forsham, P. H., Hill, S. R., Jr., Smith, S., III, and Warren, J. E.: Studies on the Relation of Pituitary-Adrenal Function to Rheumatic Disease, New England J. Med. **241**: 529, 1949.
8. Elkinton, J. R., Hunt, A. D., Jr., Godfrey, L., McCrory, W., Rogerson, A., and Stokes, J., Jr.: Effects of ACTH in Patients With Collagen and Allied Diseases, Proceedings of the First Cinical ACTH Conference, edited by J. R. Mote, Philadelphia, 1950, The Blakiston Company, pp. 429-436.
9. Elkinton, J. R., Hunt, A. D., Jr., Godfrey, L., McCrory, W., Rogerson, A., and Stokes, J., Jr.: Effects of Pituitary Adrenocorticotropic Hormone (ACTH) Therapy, J. A. M. A. **141**: 1273, 1949.
10. Ragan, C., Grokoest, A. W., and Boots, R. H.: Effect of Adrenocorticotropic Hormone (ACTH) on Rheumatoid Arthritis, Am. J. Med. **7**: 741, 1949.
11. Heller, B. I., and Jacobson, W. E.: Renal Hemodynamics in Heart Disease, Am. Heart J. **39**: 188, 1950.
12. Earle, D. P., Jr., Taggart, J. V., and Shannon, J. A.: Glomerulonephritis. A Survey of the Functional Organization of the Kidney in Various Stages of Diffuse Glomerulonephritis, J. Clin. Investigation **23**: 119, 1944.
13. Earle, D. P., Jr.: Renal Function Tests in the Diagnosis of Glomerular and Tubular Disease, Bull. New York Acad. Med. **26**: 47, 1950.
14. Talbott, J. H., Pecora, L. J., Melville, R. S., and Consolazio, W. V.: Renal Function in Patients With Addison's Disease and in Patients With Adrenal Insufficiency Secondary to Pituitary Pan-hypofunction, J. Clin. Investigation **21**: 107, 1942.
15. Waterhouse, C., and Kentmann, E.: Kidney Function in Adrenal Insufficiency, J. Clin. Investigation **27**: 372, 1948.
16. Gaudino, M., nad Levitt, M. F.: Influence of the Adrenal Cortex on Body Water Distribution and Renal Function, J. Clin Investigation **28**: 1487, 1949.
17. Ingbar, S. H., Relman, A. S., Burrows, B. A., Kass, E. H., Sisson, J. H., and Burnett, C. H.: Changes in Normal Renal Function Resulting From ACTH and Cortisone, abstracted in J. Clin. Investigation **29**: 824, 1950.
18. Perera, G. A., Pines, K. L., Hamilton, H. B., and Vislocky, K.: Clinical and Metabolic Study of 11-Dehydro-17-Hydroxy-Corticosterone Acetate (Kendall Compound E) in Hypertension, Addison's Disease and Diabetes Mellitus, Am. J. Med. **7**: 56, 1949.
19. Perera, G. A., Fleming, T. C., Pines, K. L., and Crymble, M.: Cortisone in Hypertensive Vascular Disease, J. Clin. Investigation **29**: 739, 1950.
20. Perera, G. A.: Modification of Blood Pressure by Cortisone and ACTH in Normotensives and Hypertensives, Proceedings of the First Clinical ACTH Conference, edited by J. R. Mote, Philadelphia, 1950, The Blakiston Company, pp. 284-287.
21. Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F.: Observations on the Physiologic Effects of Cortisone and ACTH in Man, Arch. Int. Med. **85**: 199, 1950.

AN ANALYSIS OF NORMAL GUINEA PIG LUNG FOR FACTORS DETERMINING PULMONARY EDEMA AND CONGESTION

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PULMONARY edema and congestion of the lungs with blood are conditions frequently observed in diseases such as left heart failure, bulbar poliomyelitis, brain injuries and lung damage due to toxic and irritant gases. The physiologic etiology of this condition is still controversial, Henneman,[1] especially where pulmonary edema and congestion follow vagal paralysis such as occurs in bulbar poliomyelitis, Reichsman.[2] One of the difficulties in studying quantitatively the factors determining pulmonary edema has been the lack of a quantitative method for determining the degree of injury in the edematous lung. Methods used by previous workers have consisted of the usual pathologic examination where the edematous lung is inspected visually to determine its appearance and color, and fixed and stained sections are studied by histologic examination. These methods while adequate for determining conditions of severe pulmonary edema are unreliable as an indication of mild or moderate edema. Furthermore, they are not numerically quantitative. In order to obtain additional quantitative information on the state of edema and congestion in extirpated lungs, a system of chemical analysis has been devised whereby the lung is analyzed for factors which change when edema develops. This system of analysis consists in determining (1) lung weight and volume, (2) insoluble protein nitrogen of the lung parenchyma which will not change during the development of edema, (3) the soluble protein nitrogen, i.e., plasma protein which accumulates in the edematous lung, (4) pulmonary hemoglobin, which measures the so-called "congestion," and (5) nonprotein nitrogen. Analyses for these nitrogen fractions have been made on more than seventy normal guinea pig lungs, and lung weight-body weight measurements have been made on more than one hundred lungs. The nitrogen fractions have been determined as functions of normal lung weights as well as body weights. The number of samples analyzed has been sufficient to determine variability by the usual methods of large-scale statistical analysis. The normal values, and their variabilities, will serve as standards with which abnormal values, obtained from animals subjected to edematous processes, can be compared.

METHODS

The guinea pigs were normal, healthy animals ranging in weight from 180 to 800 grams. They were kept in cages in the laboratory for two weeks before being sacrificed. The animals were killed by guillotine and the lungs removed immediately. The lungs were trimmed to remove adhering tissue, the large vessels were cut close to the root of the lung, the lungs were weighed and the volumes determined. The lungs were then ground in sand

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to a paste and extracted with 1 per cent sodium chloride solution. The nitrogen in the insoluble residue (including sand) was determined and designated as insoluble protein nitrogen (IPN). The nitrogen in this fraction consists of the insoluble protein forming the framework and the parenchyma of the lung. During an acute edematous process in which fluid, erythrocytes, and plasma protein accumulate in the lung this fraction should not change. The saline extract contains plasma protein and hemoglobin. Hemoglobin is determined colorimetrically by drying an aliquot of the saline extract in vacuo and extracting the dry residue with a mixture of hydrochloric acid and acetic acid (approximately each 0.1 normal) in ethyl alcohol (97 per cent) and ether in a volume ratio of 4:1. This extract, after removing the solid residue by centrifugation, is free from turbidity and the hemoglobin is determined colorimetrically as acid hematin. From another aliquot of the saline extract the soluble proteins are precipitated by tungstic acid and the soluble protein nitrogen is determined. The hemoglobin nitrogen is subtracted from this value to give the soluble (nonhemoglobin) protein nitrogen (SPN), a fraction consisting of plasma protein. After tungstic acid precipitation of the soluble protein the supernatant fluid (Folin-Wu filtrate) contains the nonprotein nitrogen (NPN) fraction. Details of this procedure are published elsewhere.[3]

RESULTS

Lung Weight.—A complete analysis of the seventy to seventy-five normal guinea pig lungs obviously cannot be given because of space limitations. The pertinent results of the analyses, which can be used by investigators studying pulmonary problems, have been represented in graphs showing the trends and variabilities and also in block diagrams which are useful for a rapid visual comparison. The pertinent statistical data are collected in a table of formulas, Table II.

The relation between lung weight (L) and body weight (B) for 103 normal guinea pigs is given in Fig. 1 with individual values being represented by points on the graph. A linear regression line has been drawn using the method of least squares. The standard error of estimate has been determined and is represented by the two outer solid lines. It was evident by inspection of the scatter diagram that the absolute variability was greater for the lungs of the large animals. It seemed advisable, therefore, to determine the variation in standard error of lung weight for animals of different weights. The method used was to divide the samples into groups according to body weights, that is, one group would include body weights of 150 to 199 grams, the second group 200 to 249 grams, and so on for the heavier animals. The standard error of estimate was determined for each group and plotted against body weight. The points obtained approximated a straight line and hence a linear regression formula was obtained, giving standard error as a function of body weight. This is represented by the two broken lines. The error determined in this way was variable and varied linearly with body weight (hence it is designated as the variable standard error of estimate). The standard error, computed in the usual manner, is a constant for all body weights.

When the lung weight/body weight ratio is plotted as a function of body weight, Fig. 1, it is evident that the ratio decreases with increasing body weight. This negative slope has been shown to be significantly different from zero. The negative slope indicates that as the animals become larger there is

not a proportionate increase in lung weight. This may be due to the fact that the heavier animals increase their weight by fat deposition and this obesity is not associated with a concomitant increase of lung weight.

Table I contains values of the lung weight/body weight ratio of normal animals obtained by other investigators who have studied pulmonary edema formation. It will be noted that there is a close agreement between the values reported in this paper and the values obtained by MacKay, Jordan, and MacKay[4] for normal guinea pigs.

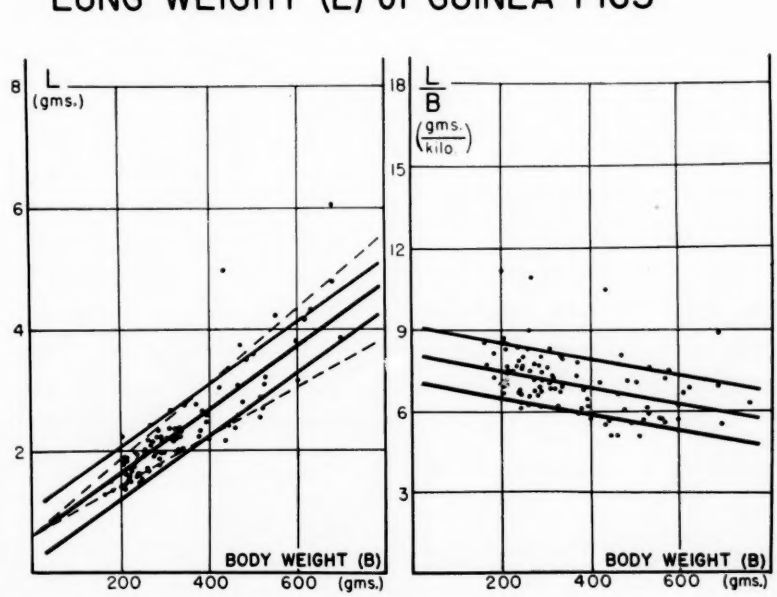

Fig. 1.

Insoluble Protein Nitrogen (IPN).—The values of insoluble protein nitrogen (IPN) for increasing weights of guinea pig lungs are given in the block diagram of Fig. 2. From this diagram a general estimate of the values and vari-

TABLE I. LUNG WEIGHT (GRAMS)/BODY WEIGHT (KILOGRAMS) OF NORMAL ANIMALS

A Comparison of Values of Normal Lung Weight/Body Weight Ratios With Values Obtained by Other Investigators for Guinea Pigs and Other Animals Used in Studies of Pulmonary Edema

ANIMALS	NUMBER OF ANIMALS	LUNG WT./BODY WT. MEAN	STAND. DEV.	MIN.	MAX.	INVESTIGATORS
Dog	54	7.96	0.80	5.4	9.7	Wood and Moe[6]
Dog	5	7.93				Eaton and co-workers[5]
Rabbit	12	4.09				Stone and Loew[7]
Rabbit	4	4.6				MacKay and co-workers[4]
Rat	6	5.3	0.6			MacKay and co-workers[4]
Guinea Pig	18	6.9	1.1			MacKay and co-workers[4]
Guinea Pig	103	7.02	1.15	4.65	10.95	Hemingway and Campbell

ability can be obtained. The more exact statistical values are given in Fig. 3 and in Table II. The variation of the values of IPN as a function of lung weight is expressed by two terms, namely, (1) a constant variation, the standard error of estimate, and (2) a variable variation term, the variable error of estimate, computed in a manner as described for lung weight and body weight.

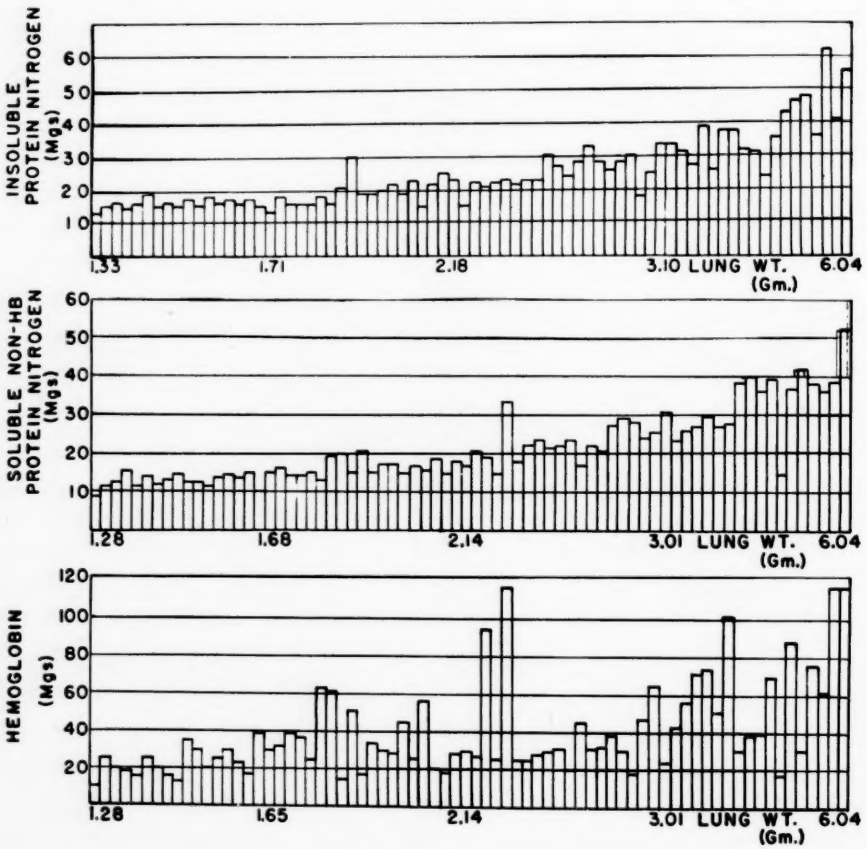

Fig. 2.

Soluble (Nonhemoglobin) Protein Nitrogen (SPN), Hemoglobin and Nonprotein Nitrogen (NPN).—The values for the soluble protein nitrogen (SPN) and hemoglobin (Hb) are given in the block diagrams of Fig. 2, where individual values are arranged in increasing order of the lung weights. The scatter diagrams of SPN, Hb, and NPN are given in Fig. 3, together with the linear regression lines and limiting lines representing the standard error of estimate and a variable error of estimate computed as described for lung weight and body weight. The *equations* of linear regression are given in Table II, together with the numerical values of the variability coefficients and the correlation coefficients.

DISCUSSION

The main purpose of this investigation was to obtain data which could be considered as standard values of chemical fractions of the normal guinea pig lung. The method of analysis was especially chosen to give analytical results which could be used in evaluating the degree of pulmonary edema. In pulmonary edema according to the usual concepts, the lung is enlarged in both volume and weight by an inflation process in which fluid accumulates in excessive amounts so that the volume of the respiratory passages is significantly

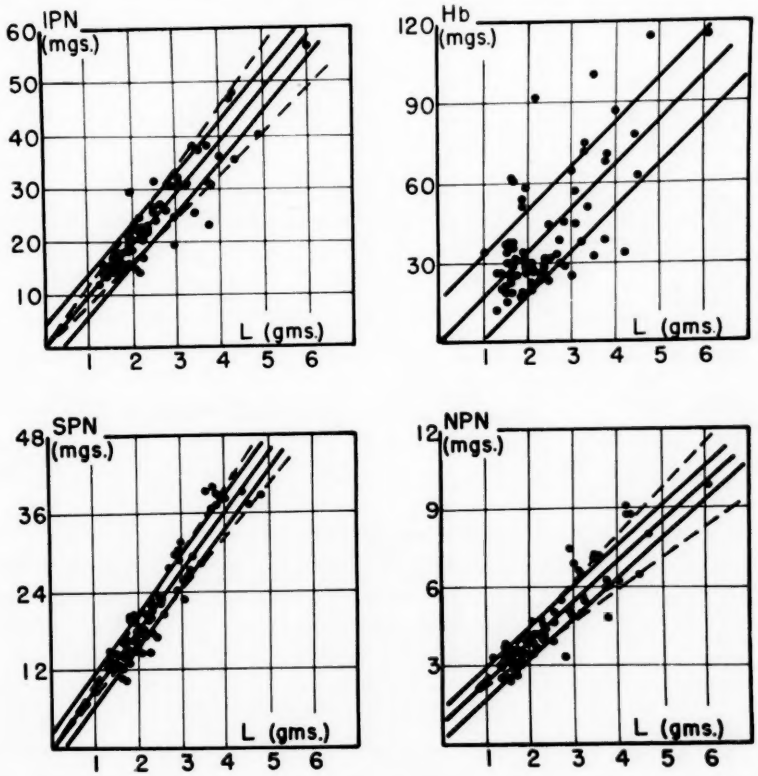

Fig. 3.

reduced either by compression or collapse of pulmonary alveoli or by the exudation of fluid into the alveoli. The fluid which accumulates in the lung is blood or a fluid derived from blood. Hence, in quantitatively evaluating pulmonary edema by chemical analysis of the edematous lung, analyses have been made for those constitutents which will increase significantly in edema. From the concept of acute edema as a swelling due to invasion by excess fluid there should not be an increase in the tissue framework and parenchyma of the lung. These contain the structural insoluble protein. The insoluble protein nitrogen should therefore furnish a measure of the lung size before the edematous process has developed.

TABLE II. MATHEMATICAL FORMULAS

Formulas for the Various Fractions as Functions of Lung Weight and Body Weight Determined by the Method of Least Squares. The Computed Errors of Variation Are Given as the (Constant) Standard Error of Estimate and the (Variable) Standard Error of Estimate.

Lung weight (L), grams, and body weight (B), kilograms. Number of animals, 103.
$L = 0.56 + 5.2B \pm 0.41$ (constant error of estimate)
$L = 0.56 + 5.2B \pm (-0.05 + 1.2B)$ (variable error of estimate)
$L/B = 8.08 - 3.0\ B \pm 1.05$
Correlation coefficient, L and B, 0.822

Insoluble protein nitrogen (IPN), milligrams, as a function of lung weight (L), grams and body weight (B), kilograms. Number of animals, 74.
$IPN = 0.097 + 9.8L \pm 4.3$ (constant error of estimate)
$IPN = 0.097 + 9.8L \pm (-0.97 + 1.78L)$ (variable error of estimate)
$IPN/L = 10.05 - 0.056\ L \pm 1.46$
$IPN = 5.13 + 52.0\ B \pm 5.8$
$IPN/B = 83.0 - 37.0\ B \pm 13.5$
Correlation coefficient, IPN and L, 0.957

Soluble protein (non Hb) nitrogen)SPN), milligrams, as a function of lung weight (L), grams, and body weight (B), kilograms. Number of animals, 73.
$SPN = -0.08 + 8.9L \pm 2.63$ (constant error of estimate)
$SPN = -0.08 + 8.9L \pm (0.98 + 0.56L)$ (variable error of estimate)
$SPN/L = 7.75 + 0.45\ L \pm 1.1$
$SPN = 4.35 + 50.0B \pm 5.0$
$SPN/B = 640.0 - 57.0B \pm 13.9$
Correlation coefficient, SPN and L, 0.928

Hemoglobin (Hb), milligrams, as a function of lung weight (L), grams, and body weight (B), kilograms. Number of animals, 73.
$Hb = -0.6 + 16.8\ L \pm 16.8$ (constant error of estimate)
$Hb = -0.6 + 16.8L \pm (5.94 + 3.61\ L)$ (variable error of estimate)
$Hb/L = 17.1 - 0.142\ L \pm 6.9$
$Hb = 9.32 + 86.0\ B \pm 19.1$
$Hb/B = 151.0 - 90.8\ B \pm 63.9$
Correlation coefficient, Hb and L, 0.688

Nonprotein nitrogen (NPN), milligrams, as a function of lung weight (L), grams, and body weight, kilograms. Number of animals, 73.
$NPN = 0.78 + 1.53\ L \pm 0.66$ (constant error of estimate)
$NPN = 0.78 + 1.53L \pm (-0.01 + 0.25\ L)$ (variable error of estimate)
$NPN/L = 2.17 - 0.11\ L \pm 0.26$
$NPN = 1.51 + 8.3\ B \pm 0.96$
$NPN/B = 17.3 - 11.3\ B \pm 2.36$
Correlation coefficient, NPN and L, 0.911

The data obtained represent analyses for weight on more than one hundred normal guinea pigs and for nitrogen fractions on more than seventy of the same animals. Some difficulty has been experienced in selecting from this mass of data and statistical calculation the most useful methods of representing the data. In considering the most general uses of this analytical data (as standards for organ analysis and pulmonary edema) the method of using block diagrams has been selected as being useful for a rapid estimate of the normal values and variations. For exact numerical standards the various nitrogen fractions have been represented as functions of lung weights and body weights by regression formulas with their variations. These are given in Table II.

Using the formula relating insoluble protein nitrogen (IPN) to lung weight, as given in Table II, it is possible to determine lung weight from IPN. The lung weight obtained in this way is the normal lung weight. If a lung is

taken from an animal with pulmonary edema the lung weight can be measured directly and compared with the theoretical lung weight to determine the degree of edema. The significance of the observation will be determined by the variability for the normal lung weight which can be obtained from the formulas of Table II, using either the standard error or the variable standard error. In using the formula this way it must apply to an experimental procedure involving acute edema and not an enlarged lung due to hyperplasia. Hyperplasia would involve an increase in the nitrogen of the tissue framework and parenchyma, i.e., the IPN.

The soluble protein nitrogen in normal lungs varies from 10 to 50 mg. and is closely correlated with lung weight. In determining excess of soluble protein in an edematous lung the normal soluble protein content can be determined from the insoluble protein nitrogen and the regression formula shown in Table II.

Pulmonary edema is almost always associated with an increase of hemoglobin in the lungs so that the lungs instead of having a "pale" normal appearance have petechial hemorrhages, patches of hemorrhage, or they may be "hepatogenous." A measure of pulmonary hemoglobin is a measure of the so-called "congestion" of lungs. The amount of hemoglobin has been determined in more than seventy normal lungs, and the values range from 10 to 120 mg. per lung approximately. There is considerable variability. The amount of hemoglobin is only poorly correlated with lung weight. This is evident from Figs. 2 and 3 where it will be observed that the hemoglobin values may be high in small lungs and low in large lungs. This is indicated by the comparatively large standard error of ±16.8 milligrams. It is of interest that in the normal lung the relation between SPN and lung weight has only a small standard error (±2.63 mg.) for good correlation, whereas with hemoglobin a greater variability is observed. Apparently the "blood" of normal lungs, after death by guillotine, will contain plasma protein equivalent to lung weight but hemoglobin only very poorly related to lung weight.

SUMMARY AND CONCLUSIONS

Normal guinea pig lungs have been analyzed for quantities which are useful in determining quantitatively the degree of pulmonary edema. In 103 guinea pigs having body weights varying from 180 to 800 grams, lung weight/body weight ratios have been obtained. Approximately seventy normal guinea pig lungs have been analyzed for (1) insoluble protein nitrogen, i.e., protein nitrogen of the tissue framework and parenchyma, (2) hemoglobin, (3) soluble (nonhemoglobin) protein nitrogen, i.e., pulmonary plasma protein, and (4) nonprotein nitrogen. Normal values of these fractions have been obtained and expressed by statistical methods as mathematical functions of lung weight and body weight. Using statistical procedures, correlation coefficients and variations have been determined.

The assistance of colleagues Margaret Murray, Melvin Witthaus, Robert Swanson, and John Schwab, who have performed these analyses, is gratefully acknowledged.

REFERENCES

1. Henneman, P. H.: Acute Pulmonary Edema With Especial Reference to Experimental Studies, New England J. Med. **235**: 590, 619, 1946.
2. Reichsman, F.: Studies on the Pathogenesis of Pulmonary Edema Following Bilateral Vagotomy, Heart **31**: 590, 1946.
3. Hemingway, A.: A Method of Chemical Analysis of Guinea Pig Lung for the Factors Involved in Pulmonary Edema, J. LAB. & CLIN. MED. **35**: 817, 1950.
4. MacKay, E. M., Jordan, M. D., and MacKay, L. L.: Experimental Pulmonary Edema. II. Pathogenesis of Pulmonary Edema Caused by the Ammonium Ion, Proc. Soc. Exper. Biol. & Med. **72**: 421, 1949.
5. Eaton, R. M., Czebrinski, E. W., and Smith, J. R.: Observations on Pulmonary Arterial Pressure and Peripheral Venous Pressure Following Arterial Blood Loss, J. Thoracic Surg. **14**: 339, 1947.
6. Wood, E. H., and Moe, G. K.: The Measurement of Edema in the Heart Lung Preparation, Am. J. Physiol. **136**: 506, 1942.
7. Stone, C. A., and Loew, E. R.: Effect of Various Drugs on Epinephrine-Induced Pulmonary Edema in Rabbits, Proc. Soc. Exper. Biol. & Med. **71**: 122, 1949.

LABORATORY METHODS

A MODIFIED DIGITONIN REAGENT FOR THE DETERMINATION OF CHOLESTEROL

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RECENTLY other methods have been proposed for the clinical determination of cholesterol[1, 2, 3] but the older method of Schoenheimer and Sperry[4] is still the most widely employed and is considered standard. Nevertheless, it is well recognized that its use is often accompanied by irregularities. These, when not due to technical variations, can frequently be attributed to the digitonin reagent used. The original directions for preparing this reagent[4] specify Hoffman-La Roche digitonin and describe the use of an aqueous solution which requires approximately two days to prepare. Subsequent directions of Sperry[5] recommend an aqueous solution prepared simply by dissolving 400 mg. of digitonin in 100 ml. of water. In the textbooks of Hawk and co-workers[6] progressive editions vacillate between the original directions for preparing this reagent and the more recent ones of Sperry.[5] We, as well as other workers,[7] have found that simple solution of 400 mg. of digitonin in 100 ml. of water, regardless of the digitonin product used, does not give reproducible results. Attempts to prepare duplicate standard curves with this reagent have met with repeated failure.

Previous experience with the method indicates that if Hoffman-La Roche digitonin is used and if the reagent is prepared according to the original directions[4] reproducible results are obtained. However, Hoffman-La Roche digitonin is not always readily available and this preparation of the reagent is a relatively long procedure. In view of these disadvantages it became necessary to modify the preparation of the digitonin reagent to meet our needs. The following data describe the experimental results obtained with the modified procedure.

MATERIALS AND METHODS

The materials and reagents are those described by Hawk and co-workers[6] with the exception of the digitonin.

The digitonin samples used are listed below:

1. Hoffman-La Roche.*
2. S. B. Penick & Co. Lot THX-1611
3. S. B. Penick & Co. Lot 971-LGB
4. Merck. Lot 41020.† Approximately 10 years old.
5. Merck. Lot 40619
6. Merck. Lot 50600

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*Kindly supplied by Dr. R. Gordon Gould, Presbyterian Hospital, Chicago.

†Kindly supplied by Dr. C. J. Farmer, Department of Chemistry, Medical School, Northwestern University.

The procedure followed for the precipitation is essentially that outlined by Hawk and associates[6] with the following three exceptions.

1. A 2 ml. volume of the solution containing cholesterol has been found most satisfactory for our needs and, therefore, this is the volume from which the cholesterol has been precipitated in standard curve runs.

2. *Alcoholic-Aqueous Digitonin Reagent:* A saturated alcoholic (absolute) solution of digitonin is prepared. The solubility of different products in alcohol varies widely, but for the samples listed 1,250 mg. per 100 ml. has been found to be adequate. To effect immediate maximum solution the flask is thoroughly shaken and placed in a water bath at 35 to 45° C. for twenty-five to thirty minutes. During this time it is thoroughly shaken at least twice. The flask is then removed from the bath and allowed to come to room temperature. If an appreciable amount of undissolved material remains after thirty minutes the solution is centrifuged and the supernatant decanted. This reagent may be kept on the shelf for several weeks as the stock digitonin solution. If, on standing, a precipitate settles out it may be centrifuged and the supernatant retained as the stock solution.

One volume of the alcoholic stock solution is diluted with exactly 2 volumes of distilled water just prior to its use. The solution is shaken gently during this addition and, finally, is thoroughly mixed. Usually a slightly turbid solution results. This is the actual precipitating reagent.

3. *Two* milliliters of the alcoholic-aqueous solution described are used for precipitating the cholesterol from 2 ml. of either the standard solution or the unknown sample.

It has been found that complete stirring with six to twelve scratch strokes on the side of the centrifuge tube promotes precipitation and facilitates constant results.

An eighteen-hour precipitation period has been found to be satisfatcory.

RESULTS

Standard curves have been prepared by precipitation of cholesterol (recrystallized twice) from solutions made with glacial acetic acid as well as from solutions prepared in acetone-alcohol (1:1) with the described digitonin reagent. The results of two different workers have been pooled and the regression line

TABLE I

WORKER	CHOL. (MG.)	OPT.DEN. AT 620 Mμ	CONDITIONS	DIGITONIN	DATE	STANDARD DEVIATION
B	0.105	0.10	All from a solution	Penick THX-1611	4/25/50	0.0048
		0.106	of cholesterol in	Hoffman-La Roche	5/10/50	
		0.093	glacial acetic acid	Merck 40619		
		0.099		Merck 50600		
		0.098		Merck 41020	5/11/50	
		0.105		Penick 971-LGB		
A	0.20	0.18	From glacial acetic	Penick THX-1611	4/21/50	0.0084
		0.20	acid	Merck 41020	4/29/50	
		0.19	From acetone-	Merck 41020	5/12/50	
		0.20	alcohol	Penick 971-LGB		
		0.195		Merck 50600		
A	0.30	0.27	From glacial acetic	Penick THX-1611	4/21/50	0.0084
		0.312	acid	Merck 41020	4/29/50	
		0.295	From acetone-	Merck 41020	5/12/50	
		0.293	alcohol	Penick 971-LGB		
		0.293		Merck 50600		
B	0.419	0.42	All from a solution	Penick THX-1611	4/25/50	0.003
		0.417	of cholesterol in	Hoffman-La Roche	5/10/50	
		0.411	glacial acetic acid	Merck 40619		
		0.417		Merck 50600		
		0.415		Merck 41020	5/11/50	
		0.416		Penick 971-LGB		

has been calculated for thirty-four points on a standard curve obtained by testing the six different lots of digitonin listed in the section on Materials and Methods. Fig. 1 illustrates the line obtained.

The regression line is expressed by the equation:

$$Y = -0.0055 + 1.0001X$$

Standard error of estimate = 0.0095

This figure for the standard error of estimate indicates that, when the optical density readings of unknowns fall in the middle range of the curve, one can anticipate an error of about 5 per cent in 68 per cent of the samples tested.

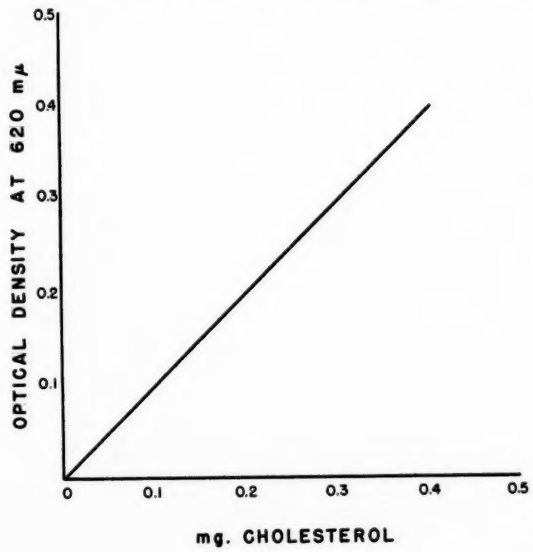

Fig. 1.

At the low end of the curve the anticipated error will be somewhat larger, while at the high end it will be smaller. Since these calculations incorporate slight variations in technique which exist between two different workers, part of the indicated error may be attributed to this factor. In addition, the figures include the small error due to the slight difference between the amount of precipitate obtained from solutions of cholesterol in glacial acetic acid and that obtained from solutions of cholesterol in acetone-alcohol.[4] That the method is capable of greater accuracy than that defined by the standard error figure given is indicated by the data in Table I.

The standard deviations shown in the table indicate a maximum expected error of 5 per cent in 68 per cent of all samples analyzed having values between 0.1 and 0.42 mg. of cholesterol. Because of the insolubility of cholesterol in serum and plasma it is customary in recovery experiments to add the increment of cholesterol to the acetone-alcohol extract of the samples being studied. Therefore, the standard curves prepared from solutions of cholesterol in acetone-alcohol represent recovery experiments. The foregoing analysis of the data presented indicates sufficient accuracy to justify the use of this procedure.

SUMMARY

A new procedure for the preparation of the digitonin reagent in the digitonin precipitation method for determining cholesterol is given. This modification of the original method combines the advantages of rapid preparation of the reagent with the interchangable use of several digitonins from different manufacturers.

ADDENDUM

After this manuscript was submitted for publication, a paper by W. M. Sperry and M. Webb (J. Biol. Chem. **187**: 97, 1950) appeared which incorporates a similar revision of the original Schoenheimer-Sperry method. Any knowledge of the pending appearance of this other report was, of course, not available to us.

REFERENCES

1. Hess, W. C.: Chromatographic Separation of Cholesterol and Cholesterol Esters in Blood, J. Lab. & Clin. Med. **32**: 1163, 1947.
2. Kochler, A. E., and Hill, E.: The Molecular Micro Distillation of Cholesterol and Cholesterol Esters, J. Biol. Chem. **179**: 1, 1949.
3. Kingsley, G. R., and Schaffert, R. R.: Determination of Free and Total Cholesterol by Direct Chloroform Extraction, J. Biol. Chem. **180**: 315, 1949.
4. Schoenheimer, R., and Sperry, W. M.: A Micromethod for the Determination of Free and Combined Cholesterol, J. Biol. Chem. **106**: 745, 1934.
5. Sperry, W. M.: Mimeographed Outline of the Directions for "The Schoenheimer-Sperry Method for the Determination of Cholesterol." Department of Biochemistry, the New York State Psychiatric Institute and Hospital, New York. Revised, April 1945.
6. Hawk, P. B., Osler, R. L., and Summerson, W. H.: Practical Physiological chemistry, ed. 12, Philadelphia, 1947, The Blakison Company, pp. 532, 1226.
7. Fry, Edith G.: Department of Physiological Chemistry, Yale University. Personal communication.

A SINGLE, SIMPLE, TURBIDIMETRIC METHOD FOR THE ASSAY OF AUREOMYCIN, CHLORAMPHENICOL, PENICILLIN, STREPTOMYCIN, AND TERRAMYCIN IN CAPILLARY BLOOD AND OTHER BODY FLUID

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A TURBIDIMETRIC method of aureomycin assay already has been reported by us.[1] It is a modification of a basic assay technique worked out by Osgood and Graham.[2] Its advantages are that it is technically simple; it measures accurately low concentrations of the antibiotic in the presence of human serum, urine, and cerebrospinal fluid; enough blood for assay with this method can be obtained from a single finger or heel puncture; and results can be obtained within five hours of collection of specimens. Since our previous report we have found the method to be satisfactory for the assay of chloramphenicol, penicillin, streptomycin, and terramycin under the same conditions of assay. The purpose of this report is to describe the technique and accuracy of the method when used to assay these four antibiotics.

DETAILS OF TECHNIQUE

The materials needed† and the method of calculating the results are the same as for aureomycin assay; the technique differs only in the concentration of chloramphenicol, penicillin, streptomycin, and terramycin in the physiologic saline added to the four standard tubes which are used to make up the standard curves (see Table I for this information).

RESULTS

The results of 146 consecutive determinations of known concentrations of chloramphenicol intermediately spaced between the values used to make up the standard curve are tabulated in Table II. Data from the same experiments for penicillin are in Table III, for streptomycin in Table IV, and for terramycin in Table V. Figs. 1, 2, 3, and 4 illustrate how the experiments were run and the type of standard curve obtained when the results are plotted on semilog paper. Table VI gives the range and average per cent error for the assay of

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The chloramphenicol, which has the proprietary term Chloromycetin, used in this study was supplied by Parke, Davis & Company. The terramycin was supplied by Chas. Pfizer & Co., Inc.

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†By using microcolorimeter tubes the volume of unknown specimen required for assay can be reduced below 0.5 milliliter. F. D. A. strain 209 is the *Staphylococcus aureus* used for penicillin assay.

each antibiotic in each type of body fluid, these data having been computed from the data in Tables II, III, IV, and V. The figures for aureomycin from our previous paper[1] are included in Table VI for comparison. Concentrations per milliliter as low as 1 γ of chloramphenicol, 2 γ of streptomycin, 0.025 unit of penicillin in serum and cerebrospinal fluid, 0.10 unit of penicillin in urine, 0.07 γ of terramycin in serum, and 0.31 γ of terramycin in urine and cerebrospinal fluid can be detected accurately (Figs. 1, 2, 3, 4, and Tables I, II, III, IV, and V). Lower concentrations of all four antibiotics are detectable, but not uniformly or accurately,* and are not included in the data presented here. To detect concentrations per milliliter of greater than 32 γ of chloramphenicol, 25 γ of streptomycin, 2.0 units of penicillin in serum, 3.2 units of penicillin in urine, 0.80 unit of penicillin in cerebrospinal fluid, 2.5 units of terramycin

TABLE I. CONCENTRATION OF CHLORAMPHENICOL, PENICILLIN, STREPTOMYCIN, AND TERRAMYCIN IN THE PHYSIOLOGIC SALINE ADDED TO THE STANDARD TUBES

STANDARD TUBE	CHLORAMPHENICOL (γ/ML.)	PENICILLIN (UNITS/ML.)	STREPTOMYCIN (γ/ML.)	TERRAMYCIN (γ/ML.)		
				SERUM	URINE	CEREBROSPINAL FLUID
1	1	0.025‡	2	0.10	0.31	0.40
2	3	0.10	5	0.31	0.62	0.80
3	8	0.40	10	0.62	1.25	3.20
4	32	0.80	20	1.25	2.50	25.60
5	—	1.60†‡	—	2.50	10.00	—
6	—	3.20*†	—	—	—	—

*Not included in serum standard curves.
†Not included in cerebrospinal fluid standard curve.
‡Not included in urine standard curves.

TABLE II. RECOVERY OF CHLOROAMPHENICOL FROM SERUM, URINE, AND CEREBROSPINAL FLUID

RECOVERED CONCENTRATION (γ/ML.)	KNOWN CONCENTRATION (γ/ML.)										
	1.5	2.0	4.0	5.0	6.0	7.0	10	12	14	16	24
Serum	1.4	1.7	3.6	3.8	4.3	7.2	10	13	13	20	24
	1.4	1.8	3.6	3.8	4.8	8.0	12	13	15	22	26
	1.2	2.0	3.0		6.0			13		16	29
	1.2	2.0	3.2		6.5			13		17	34
	1.2	1.8	4.2		5.9			12		17	26
	1.1	1.8	3.4		3.8			11		17	25
			3.4		4.1			11		18	27
			3.3		4.2			11			
			3.7		6.5			12			
Urine	1.6	1.4	3.5		4.0			14		21	26
	2.0	1.9	4.0		4.2			16		21	27
	1.0	2.0	3.3		5.2			14		15	27
	1.3	2.0	3.5		5.5			14		18	27
	1.5	2.0	3.4		4.0			13		17	22
	1.5	2.0	3.9		4.1			14		19	24
Cerebrospinal fluid	1.4	2.6	3.6		4.4			12		16	20
	1.6	3.0	3.8		5.0			14		16	22
	1.3	2.3	3.4		4.6			18		21	27
	1.7	3.2	3.7		5.3			18		25	29
	1.1	1.4	3.1		3.6			13		22	30
	1.1	1.4	3.1		4.1			18		22	30

*Lower concentrations of antibiotic can be detected accurately by increasing the relative volume of unknown specimen in the assay tubes.

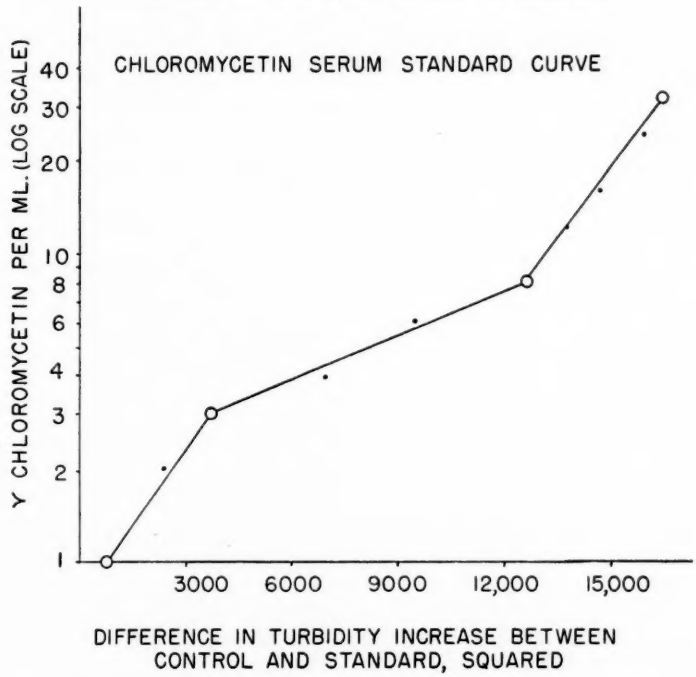

Fig. 1.—The four standard points are plotted as circles; the simultaneously determined intermediate points, as small dots.

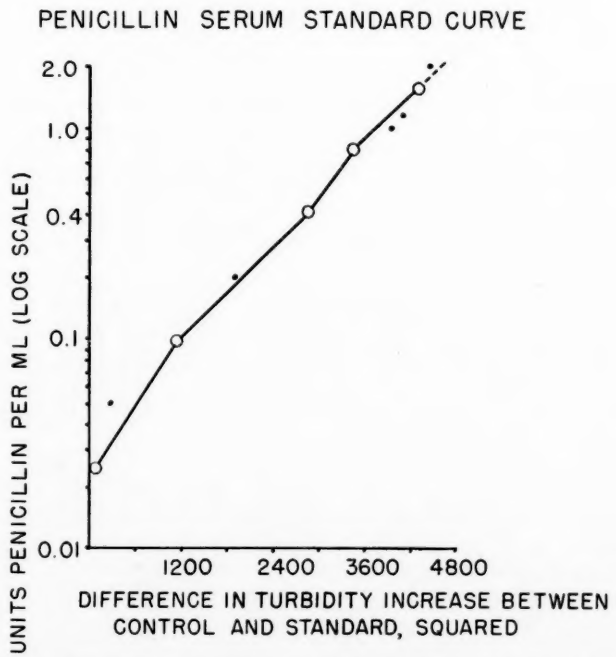

Fig. 2.—The four standard points are plotted as circles; the simultaneously determined intermediate points, as small dots.

in serum, 10.0 units of terramycin in urine, and 25.6 units of terramycin in cerebrospinal fluid, unknown specimens must be prediluted before assay (Figs. 1, 2, 3, 4, and Tables I, II, III, IV, and V), as previously described.[1] It is suggested that chloramphenicol serum specimens be prediluted 1:2 to detect 2 to 64 γ per milliliter and urine specimens be prediluted 1:5 to detect 5 to 160 γ per milliliter.

TABLE III. RECOVERY OF PENICILLIN FROM SERUM, URINE, AND CEREBROSPINAL FLUID

RECOVERED CONCENTRATION (γ/ML.)	KNOWN CONCENTRATION (UNITS/ML.)									
	0.05	0.12	0.15	0.20	0.30	0.50	0.60	1.0	1.2	2.0
Serum										
	0.030			0.19				1.2	1.4	1.8
	0.036			0.23				1.2	1.4	2.1
	0.034			0.20				1.1	1.3	1.6
	0.036			0.22				1.3	1.3	1.6
	0.034			0.14				0.78	0.98	1.4
	0.036			0.17				0.78	0.98	1.8
Urine										
		0.11		0.20	0.24	0.40		1.1		1.8
		0.10		0.21	0.25	0.36	0.80	0.80		1.8
		0.20		0.21		0.38		0.97		2.2
		0.20				0.75		0.87		2.2
Cerebrospinal fluid										
	0.053	0.16			0.25	0.63				
	0.057	0.19			0.27	0.70				
	0.042	0.11			0.25	0.69				
	0.050	0.14			0.29	0.69				
	0.042	0.27			0.36	0.58				
	0.042	0.27			0.36	0.58				

TABLE IV. RECOVERY OF STREPTOMYCIN FROM SERUM, URINE, AND CEREBROSPINAL FLUID

RECOVERED CONCENTRATION (γ/ML.)	KNOWN CONCENTRATION (γ/ML.)									
	3.0	4.0	6.0	7.5	8.0	12.5	13	15	17	25
Serum										
	2.6	4.4	5.9	6.9	6.8	13	14	17	18	23
	2.8	4.6	6.1		6.8		16		19	23
	2.7	3.7	5.8		8.0		12		20	23
	2.7	3.7	6.2		8.3		12		20	23
	2.5									21
	2.5									
Urine										
	3.6	5.1	9.5		9.5		17		17	24
	3.6	5.4	9.5		9.5		17		17	24
	2.5	4.1	6.2		9.3		15		18	20
	2.6	3.5	5.7		8.8		15		18	21
	3.0	5.0	8.6		10		25		25	32
	3.5	5.3	9.2		10		25		25	32
Cerebrospinal fluid										
	2.8	3.2	6.8		8.2		12		20	23
	2.8	3.7	7.5		9.1		16		20	23
	3.1	5.1	8.0		8.8		18		20	21
	3.3	5.2	8.8		9.1		18		20	22
	2.1	3.3	9.2		9.2		14		15	30
	2.4	3.4	9.2		9.2		14		15	30

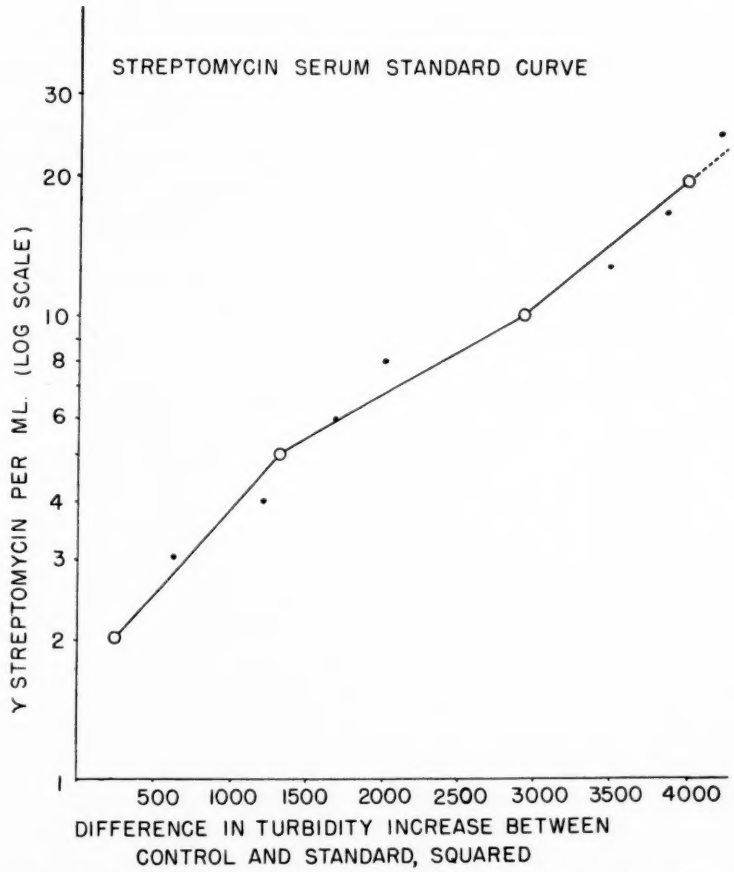

Fig. 3.—The four standard points are plotted as circles; the simultaneously determined intermediate points, as small dots.

TABLE V. RECOVERY OF TERRAMYCIN FROM SERUM, URINE, AND CEREBROSPINAL FLUID

RECOVERED CONCENTRATION (γ/ML.)	KNOWN CONCENTRATION (γ/ML.)												
	0.07	0.15	0.20	0.31	0.40	0.62	0.80	1.2	1.6	2.5	3.2	5.0	6.4	15
Serum														
	0.08	0.11	0.24		0.51		1.2		1.6					
	0.09	0.14	0.26		0.51		1.2		1.9					
	0.08	0.09	0.13		0.31		0.76		1.6					
	0.08	0.11	0.16		0.37		0.79		2.0					
	0.08	0.12	0.18		0.41		0.78		2.0					
	0.09	0.12	0.20		0.44		0.82		2.0					
Urine														
					0.32		0.80		1.5		3.4		6.4	
					0.32		0.86		1.5		3.4		8.0	
					0.34		0.80		1.5		5.0		4.8	
					0.34		0.80		1.5		5.0		4.8	
					0.32		0.67		1.4		2.5		3.5	
					0.32		0.73		1.5		3.4		7.0	
Cerebrospinal fluid														
				0.35		0.46		1.4		3.2		4.3		15
				0.35		0.46		1.4		3.2		4.3		15
				0.31		0.40		0.98		2.6		4.0		20
				0.31		0.40		0.98		2.6		4.6		20
				0.27		0.46		0.97		1.9		3.2		18
				0.28		0.52		0.97		2.1		3.2		18

TABLE VI. PER CENT ERROR FOR THE ASSAY OF AUREOMYCIN, CHLORAMPHENICOL, PENICILLIN, STREPTOMYCIN, AND TERRAMYCIN IN HUMAN BODY FLUIDS

	PER CENT ERROR					
	SERUM		URINE		CEREBROSPINAL FLUID	
	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE
Aureomycin	8	0-35	12	0-42	12	0-35
Chloramphenicol	13	0-42	14	0-35	24	0-60
Penicillin	19	0-40	20	0-66.5	20	3.5-86.5
Streptomycin	9	0-20	25	0-92	19	2-53
Terramycin	19	0-50	16	0-56	18	0-36

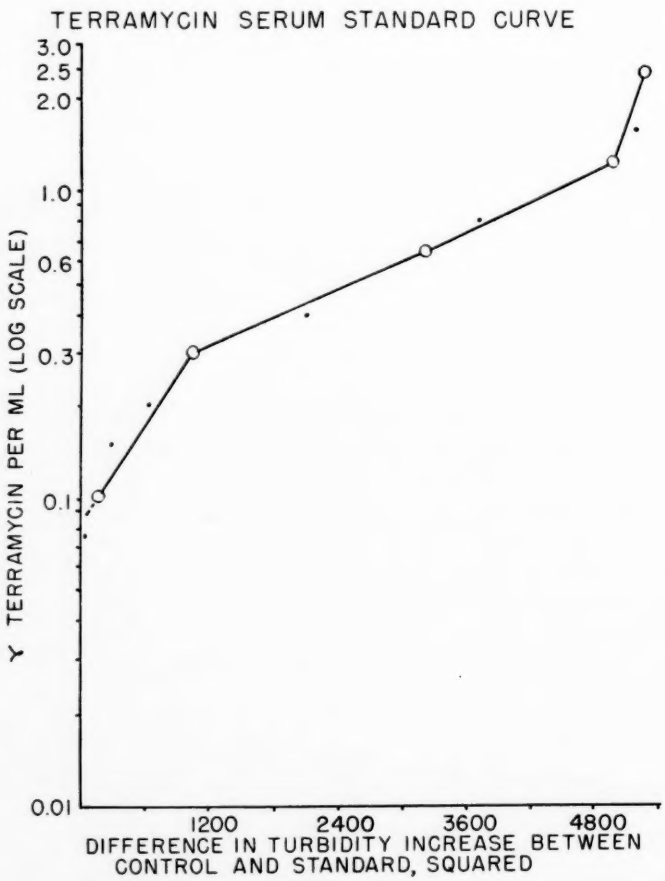

Fig. 4.—The four standard points are plotted as circles; the simultaneously determined intermediate points, as small dots.

SUMMARY AND CONCLUSIONS

A turbidimetric method of aureomycin assay has been found satisfactory also for the assay of chloramphenicol, penicillin, streptomycin, and terramycin. It appears to provide a simple, rapid and accurate means of assaying low concentrations of these five antibiotics in small volumes of various human body fluids.

REFERENCES

1. Whitlock, C. M., Jr., Hunt, A. D., Jr., and Tashman, S. G.: A Simplified Turbidimetric Method of Aureomycin Assay for Capillary Blood and Other Body Fluids, J. LAB. & CLIN. MED. **34:** 1682, 1949.
2. (a) Osgood, E. E., and Graham, D. M.: A Single Rapid Method for Assay of Bactericidal and Bacteriostatic Agents, Am. J. Clin. Path. **17:** 93, 1947.
   (b) Osgood, E. E.: Assay of Penicillin, Streptomycin, Trivalent Organic Arsenicals, and Other Bactericidal Agents, J. LAB. & CLIN. MED. **32:** 446, 1947.

A SIMPLIFIED, CONTINUOUS-FLOW APPARATUS FOR USE IN TISSUE CULTURE

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THE apparatus described is a modification of the apparatus constructed by Audus[1] for soil perfusion studies. It is well adapted for tissue culture work and is applicable to the study of many phases of cell metabolism, and to the study of the biochemistry and physiology of the pathogenesis of many virus diseases. The apparatus is simply constructed at low cost and studies can be made for long periods of time without disturbing the tissue. There are also facilities for changing the medium and for sampling the metabolic products.

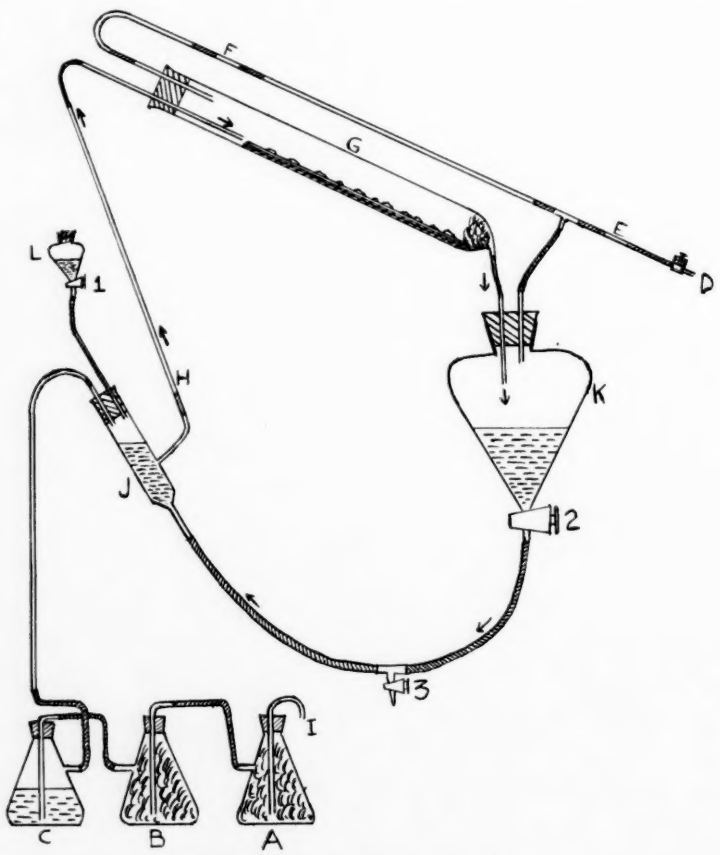

Fig. 1.—Tissue culture apparatus.

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The tissue culture apparatus was assembled as shown in Fig. 1. The air filter consisted of three 500 ml. filter flasks. Flasks *A* and *B* contained glass wool and flask *C* contained 200 ml. of nutrient broth to detect possible bacterial contamination from the air. This air filter was sterilized as a unit and attached to the tissue culture apparatus after it had cooled.

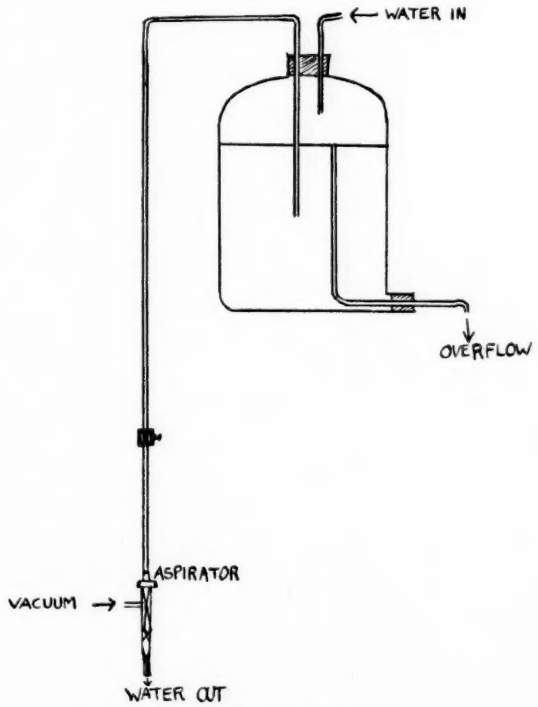

Fig. 2.—Constant-flow device.

Beginning at *D*, a constant, low vacuum was applied by means of the constant flow device (Fig. 2), which provided a convenient means for a moderate and constant vacuum. This vacuum was transmitted through two capillary tubes, *E* and *F*, and the tissue tube *G* to the nutrient solution in the feed tube *H* which had a critical internal diameter of 4 millimeters. This vacuum caused air, which entered through the air intake *I*, to be drawn in at the flow feed *J*, thereby detaching a column of solution which was drawn up the feed tube *H*. The flow feed *J* can be constructed from the neck of a distilling flask. On release of the tension in the feed tube *H* by the discharge of this column of solution into the tissue tube *G*, the solution again arose above the base of the flow feed *J* until the level in *H* reached the level of the solution in the 1,000 ml. separatory funnel *K*. More air was drawn in again at the flow feed *J* through the air intake *I* and the whole process was repeated. The liquid thus discharged in the tissue tube *G* was drawn in with the air stream through the tube and drained back into the separatory funnel *K*.

The tissue tube *G* was approximately 10 in. long with an internal diameter of 1 inch. All other glass tubing, except the feed tube *H*, had an internal diameter of 5 millimeters. The rubber tubing used in the apparatus had walls approximately 3 mm. thick. Before use its interior was scrubbed with soap and water and soaked in triple-distilled water overnight.

With all stopcocks closed, 175 ml. of nutrient broth, consisting of 3 per cent Bacto-beef extract, 5 per cent proteose peptone, Difco, and 1 per cent Bacto-yeast extract, dissolved in triple-distilled water and adjusted to pH 7.6, were then introduced through the top of separa-

tory funnel *K* and the top was securely replaced. All stopcocks were secured with wire to prevent loosening during the sterilization process, and the apparatus was sterilized for twenty minutes at 15 pounds pressure.

After cooling, 100 ml. of sterile Tydrode's solution[2] were aseptically introduced through the 200 ml. separatory funnel *L* and stopcocks *1* and *2* opened in order to allow the Tyrode solution to mix with the nutrient broth in separatory funnel *K*. Simultaneously a slight vacuum, which can be controlled with the screwclamp on the outlet from the constant flow device, was applied to the apparatus to ensure thorough mixing.

A mixture of 25 ml. of sterile cattle serum and 100 ml. of sterile chick embryo extract[2] was then introduced aseptically through separatory funnel *L*, and after sufficient mixing a sample of the resulting medium was taken by opening stopcock *3* and the pH of the solution was tested. If the pH was not within the range of 7.5 ± 0.1, it was adjusted by adding aseptically, through separatory funnel *L*, sterile 0.1N acid or base which was then washed down with a small amount of sterile physiologic saline.

Twenty thousand units (50 units per milliliter of medium) of crystalline penicillin G* were then added aseptically from a syringe to the medium through separatory funnel *L*. It was allowed to circulate and mix thoroughly and the vacuum was then discontinued while closing stopcocks *1* and *2* and plugging separatory funnel *L* with sterile cotton.

Chick embryo tissue was used in this investigation. Eleven-day old fertile hens' eggs were disinfected with tincture of iodine and alcohol and the embryos removed and washed in sterile Tyrode's solution. The head and legs were removed because of their interference in maceration.

While the embryo was being washed, a sterile mixture of 10 ml. of 3 per cent Bacto-beef extract, 5 per cent proteose peptone, 1 per cent Bacto-yeast extract, 2 per cent Bacto-agar dissolved in triple-distilled water, adjusted to pH 7.6, was liquified and cooled to 40° C. After 1 ml. of sterile cattle serum was added aseptically, the mixture was put into the tissue tube *G* on stainless steel gauze which was used as a support. While this mixture was still semisolid, the tissue to be grown was placed in a sterile 10 ml. syringe without a needle and shot onto this tissue support, and the end of the tube was flamed and closed.

The apparatus was placed in an incubator at 37° C. The vacuum was continued after the agar had solidified, the tissue being continuously supplied with nutrients by means of the cycle already described.

Emphasis is placed on employing aseptic techniques throughout this procedure, keeping the pH constant, and also using the slightest vacuum applicable to the satisfactory performance of the apparatus.

A period of three or four days must elapse before growth can be detected. The naked eye, or better, a magnifying glass may be used to observe the outgrowths of fibroblasts around the edges of the embedded tissue.

REFERENCES

1. Audus, L. J.: A New Soil Perfusion Apparatus, Nature **158**: 419, 1946.
2. Parker, R. C.: Methods of Tissue Culture, New York, 1938, Paul B. Hoeber, Inc.

*Supplied through the courtesy of Commercial Solvents Corporation.